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N. N. Sirotinin et al.

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16. Abstract The present collection discusses the principal extremal effects to which astronauts may be subjected during space flight: depressurization and decompression (amounting in the main to anoxia), gravitation and weightlessness, hypokinesia and kinetosis. The prophylaxis and therapy of these conditions are indicated. Also described are how to provide astronauts with water that is regenerated and preserved under spacecraft conditions and how to cultivate algae that can serve as a source of oxygen and food. The book is addressed to biologists and physicians who are interested in space flight.

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EXPERIMENTAL APPLICATION OF THE ARTIFICIAL BLOOD CIRCULATION METHOD AND THE TANK METHOD FOR THE REANIMATION OF DOGS AFTER CLINICAL DEATH FROM ACUTE DECOMPRESSION

N. N. Sirotinin, V. D. Yankovskiy, and Yu. F. Gerya

The two groups of experiments on the reanimation of dogs that had succumbed to acute anoxia, the results of which are presented below, are a continuation of the studies of N. N. Sirotinin et al. In the first group of experiments, to reanimate the animals the artificial blood circulation method was employed. In the second group of experiments, the dogs were revived by means of a new tank method of reanimation, which has recently been worked out in our laboratory. /3*

First group of experiments. Of the 34 experiments of the first group, 11 were conducted with the aid of the SB-3 autojector and S.S. Bryukhonenko's artificial blood circulation method with aeration of the blood in artificial lungs of the foam type designed by V. D. Yankovskiy and S. S. Bryukhonenko; this constitutes the first subgroup of experiments. In the rest of the experiments -- the second subgroup -- the dogs were revived by the donor method (a variant of Bryukhonenko's artificial blood circulation method).

The data from the first group of experiments, which describe changes in some of the organism's physiological functions at the time of death and during resuscitation, are shown in the table below. We established that incident to clinical death from acute anoxia, the agony of the dogs was brief: from 1.5 to 4.5 min. In half of the cases, the dogs were revived, but only two of them, which had undergone clinical death lasting 10.5 and 18 min (experiments 803 and 812), survived for a protracted period of time; these were revived by means of Bryukhonenko's classical artificial blood circulation method. The rest of the experiments, which are not included in the table, took more or less the same course.

The experiments of the first group were conducted in the following manner. The autojector and artificial lung system were filled beforehand with mutually compatible donor blood. As anticoagulants we used heparin or sinantrin (similar to heparin) prepared at the Kharkov Medicinal Preparations Plant by our Institute's method. One of these preparations was introduced into the dogs intravenously in doses of 500-600 IUA (Internal Unit of Activity) per kg of body weight, which is customary in the case of

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* Numbers in the margin indicate pagination in the foreign text.

TABLE 1. RESTORATION OF IMPORTANT VITAL FUNCTIONS IN DOGS THAT HAD SUCCUMBED TO ACUTE ANOXIA (FIRST GROUP OF EXPERIMENTS)

Experiment No.	Body weight, kg	Rarefaction effected: For a time of, sec	Rarefaction effected: To an altitude of, (km)	Duration: Of stay in altitude chamber	Duration: Of return to normal pressure, sec	Duration: Of agony	Duration: Of clinical death	Duration: Of absence of respiration	Restoration from start of reanimation: Of respiration	Restoration from start of reanimation: Of heart action	Duration: Of artificial blood circ., min	Duration: Of cross-transfusion, min	Duration: Of second life
First subgroup of experiments*													
791	3.5	—	—	—	—	—	11 min 30 sec	18 min 21 sec	6 min 51 sec	15 min 21 sec	18	—	10 hr
800	2.6	70	>20	3 min 00 sec	30	2 min 30 sec	19 » 30 »	30 » 00 »	10 » 30 »	10 » 30 »	75	—	—
802	5.0	115	21	5 » 30 »	50	3 » 30 »	20 » 00 »	27 » 30 »	11 » 00 »	—	121	—	—
803	5.7	90	22	1 » 30 »	30	1 » 30 »	10 » 30 »	20 » 50 »	7 » 15 »	3 » 12 »	15	15	Protracted survival
804	4.5	51	23	5 » 40 »	20	3 » 40 »	17 » 05 »	21 » 45 »	4 » 45 »	12 » 39 »	36	20	14 hr
805	1.6	50	>22	6 » 00 »	45	4 » 30 »	13 » 40 »	18 » 10 »	4 » 30 »	6 » 36 »	30	—	—
809	4.2	45	>20	3 » 00 »	37	1 » 30 »	11 » 50 »	16 » 20 »	4 » 30 »	16 » 57 »	29	14	35 hr
810	3.3	105	25	6 » 20 »	45	3 » 05 »	15 » 06 »	44 » 51 »	29 » 45 »	—	48	18	—
812	3.4	60	23	3 » 00 »	30	1 » 40 »	18 » 00 »	26 » 12 »	8 » 12 »	8 » 12 »	56	10	Protracted survival
813	5.9	40	23	3 » 00 »	30	1 » 50 »	19 » 34 »	27 » 07 »	7 » 33 »	9 » 30 »	100	—	—
814	4.8	50	23	3 » 19 »	35	2 » 19 »	15 » 08 »	29 » 59 »	14 » 51 »	6 » 00 »	89	—	—
Second subgroup of experiments**													
798	2.6	80	>20	4 » 30 »	30	2 » 30 »	15 » 19 »	23 » 07 »	7 » 48 »	—	76	—	2 hr
806	5.0	40	23	3 » 13 »	25	3 » 13 »	16 » 25 »	22 » 25 »	6 » 00 »	1 » 36 »	95	17	—
807	2.9	50	>20	4 » 45 »	30	2 » 00 »	15 » 11 »	32 » 29 »	15 » 18 »	3 » 48 »	42	—	8 hr
808	3.9	45	24	3 » 00 »	35	3 » 20 »	17 » 13 »	30 » 34 »	13 » 21 »	—	69	—	—
811	5.5	45	>23	3 » 34 »	15	2 » 34 »	15 » 20 »	20 » 44 »	5 » 24 »	17 » 00 »	81	—	55 min

* Resuscitation by means of S. S. Bryukhonenko's artificial blood circulation method (blood aeration in artificial lungs).

** Resuscitation by the donor method.

heparin. In some cases, the organism of the just revived dog was "flushed" (usually for 10-20 min) during the course of a general blood transfusion by means of cross-circulation with another dog in accordance with the chain: donor artery → donee vein; donee carotid artery → donor vein. All experiments were conducted under morphine-nembutal narcosis. During resuscitation, the rate of the extracorporeal blood circulation was periodically measured, and the ECG was recorded, as well as the blood pressure and respiration (on a kymograph).

For the experiment we chose dogs of both sexes weighing 1.6-5.9 kg, each of which we placed in a 21-cm-diameter and 52-cm-long altitude chamber. Decompression was effected for 40 to 115 sec until a rarefaction from 41 to 18 mm Hg (until "ascent to an altitude of" 20-25 km). During the dog's stay in the altitude chamber, we recorded its respiration and ECG on an encephalograph. After its agony, which lasted from 1 min 30 sec to 4 min 30 sec, the dog in most cases remained in the altitude chamber with rarefied air for another 2.5-3 min. Then the pressure in the chamber was returned to normal in the course of 10-50 sec, and the chamber was opened. Into the vessels that had been prepared beforehand (jugular vein, femoral and carotid arteries), cannulas were inserted, after which the dog's vascular system was hooked up with the artificial blood circulation system. All of this took 8-15 min.

Clinical death lasted from 10.5 to 22 min -- long enough for an astronaut, in the event of the depressurization of his space-suit in open space, to be pulled back into his spacecraft and for reanimation to be started. /5

Before the start of artificial blood circulation, with the aid of the autojector pump a rather considerable amount of blood, which contained a great number of gas bubbles that had evolved during decompression, was pumped out of the organism to be resuscitated through a cannula, the lower end of which was situated in a vein near the right atrium cordis, and sucked into the artificial lungs (or donor vein). At the start of artificial blood circulation, too, gas bubbles could be observed in the venous blood. These were caught in a gas trap and did not get into the artificial lungs (or the donor's venous system), nor, a fortiori, into the arterial system of the organism to be resuscitated.

We strove to carry out reanimation under conditions of accelerated extracorporeal blood circulation (up to 180 ml blood per min per kg body weight). We now have grounds to assume that, in cases of death from acute anoxia, at the start of reanimation, when the blood vessels still contain a great amount of gas bubbles, such intensive artificial blood circulation need not be resorted to.

In the beginning of most cases of reanimation, we could observe the signs of resumed respiration: a twitching of the cervical respiratory muscles; then, rare and shallow inspirations, which were replaced by more frequent and very deep inhalations; these were succeeded by double inspirations; then, group inspirations, which were sometimes replaced by periodic breathing. Gradually, respiration took on a normal character. In some of the dogs to be resuscitated, breathing was not resumed in spite of all the measures we took -- probably because of gas embolisms in the region of the respiratory center. At first it was rare for proper heart beating to appear, and then the ECG showed monophasic complexes. In the case of most of the dogs to be resuscitated, fibrillations of the heart set in from the very start of artificial blood circulation, which were eliminated with the aid of a Gurvich-Akopyan defibrillator, after the resumption of respiration. But in some experiments, in spite of repeated (up to seven times) defibrillator shocks, we did not succeed in eliminating the fibrillations of the heart. In three cases, we did not strive for resumption of either proper respiration or heart beating in the dogs, and in these cases, autopsy established that many of their blood vessels were filled with a froth of fine bubbles.

The ocular reflexes of the resuscitated dogs appeared relatively late (after 30-40 min). The entire process of reanimation lasted from 50 min to several hours.

Autopsy of some of the dogs that died after reanimation disclosed gas bubbles in the venous and cerebral vessels. A large amount of gas was sometimes found in the pericardium and under the dura mater, and in these cases, when the dog's opened head was lifted, it could be seen how the gas bubbles moved in and out between the convolutions of the brain. In the peritoneal cavity of those dogs that were not taken from the table alive, a great amount of blood was found, and blood had effused into the brain, the muscle of the heart, the kidney and other organs.

General result: of the 34 dogs of the first group that succumbed to acute anoxia, we resuscitated by the artificial blood circulation method and kept alive only three, which had undergone clinical death for 10.5 min (Seryy), 17 min 34 sec (Belka[1]) and 18 min (Luna[2]).

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[1] Belka was resuscitated by means of the donor method (not shown in table).

[2] Luna was resuscitated on April 5, 1966. In May of the same year she was exhibited at the Second and in June of 1969, at the Third All-Union Conference on Aerospace Medicine. Our Institute donated Luna to the K.E. Tsiolkovskiy Museum in Kaluga, where she is still to be found.

It should be noted that the postreanimation period did not take a very easy course in the case of any of the long-surviving dogs. Restoration of the main functions of their central nervous systems set in only after several months had elapsed, particularly in the case of Luna (4-6 months later). After reanimating these dogs, we took all three of them up Mount Elbrus, where they were subjected to gradual acclimatization to ascending altitudes (2100, 3000, 3400 and 4200 m). What prompted us to do this was our observations of mental patients who had been subjected to this same acclimatization. As a result of their 1.5-month-long stay in the mountains, all pathological signs vanished from the dogs, and soon their behavior could not be distinguished from that of other, control dogs; they had normal litters and served duly as watch dogs. /6

Second group of experiments. Let us move on to a description of our experiments with the tank method of reanimating dogs that had died from acute decompression. In our opinion, these experiments are of indubitable scientific interest and, moreover, give grounds for hoping that the tank method, owing to its simplicity and practicability, will in the very near future be employed in the clinical practice of space medicine, as well as other branches of medicine.

As was mentioned above, the blood vessels of dogs that had died from acute decompression and were immediately removed from the altitude chamber always contained a great amount of gases in the form of bubbles of different sizes. We came to the conclusion that for the successful reanimation of these dogs, it is necessary in the first place to remove, insofar as possible, all gas bubbles from their sanguiferous system. To achieve this, we used two methods. The first was more or less as follows. After the dogs have undergone clinical death from acute decompression, the pressure in the altitude chamber is returned to normal in the course of a few seconds. After removing from the altitude chamber a dog to which heparin was immediately or previously administered intravenously, for a few minutes we perform artificial respiration and indirect massage of the heart by squeezing the thoracic cage with the hand. The dog is returned to the altitude chamber, where for a few minutes it is subjected at first to air pressure, and then to oxygen pressure (up to an overpressure of 2 atm).

By way of example, let us quote a passage from the record sheet of experiment No. 863 of May 29, 1969. The adult male dog Bobik weighing 5700 g, under morphine-nembutal narcosis. An amount of 5700 IUA heparin administered intravenously. Dog placed in altitude chamber and at 12 hours 30 min rarefaction begun. Respiratory standstill after 2 min 30 sec in a rarefied atmosphere equal to 22 mm Hg (altitude of 24 km); after 4 min from the start of the "altitude ascent," the pressure in the altitude chamber amounted to 18 mm Hg (altitude of about 25 km). "Descent" begun at 12 hours 35 min. So the dog was in the altitude chamber under

a reduced pressure about 5 min, in the course of which it was at an "altitude" of 24-25 km for 2.5 min. Upon removal from the altitude chamber, the dog did not breathe; there was no corneal reflex, but weak heart activity could be detected. For 3 min artificial respiration was performed by pressing the thoracic cage with the hand. The dog did not breathe and was therefore placed in the altitude chamber, where an air pressure of about 1 atm was created, after which the air was replaced by oxygen and the overpressure in the altitude chamber was brought up to 2 atm. After 15 min, the pressure in the altitude chamber was brought back to normal. After the dog's removal from the altitude chamber, it was established that it breathed and that its heart action had improved considerably.

In the postreanimation period, for a space of 2-3 days, the dog was sluggish; he accepted food unwillingly; he spent a great part of the time lying down. On the fourth day, the revived dog's behavior did not differ essentially from its behavior before the experiment.

Subsequently this method was somewhat improved: artificial respiration and indirect massage of the heart after the dog's death from acute decompression was accomplished without removing the dog from the altitude chamber. For this purpose, we designed a special device called an automatic regulator of artificial respiration, which consists of a rubber cuff that can be applied to the thoracic cage before the dog is placed in the altitude chamber, and an electromechanical inspiration-expiration regulator. A metallic belt was mounted above the cuff, so that the air pressure created in it periodically squeezed the thoracic cage (inspiration), which was followed by inspiration, when the air was removed from the cuff.

The experiments were conducted in the following manner. The /7
atmosphere in the altitude chamber in which the dog was placed was rarefied down to 30.3-18.9 mm Hg ("altitude" of 22 to 25 km). After 3-5 min, air was fed into the chamber up to the normal pressure, after which air from a cylinder was introduced into the chamber up to an overpressure of 2 atm. One-two min thereafter, we performed a few minutes of artificial respiration and indirect massage of the heart with the aid of the above-described device. Respiration was usually restored during "descent," but, in a number of cases, we were obliged to have additional recourse to artificial respiration with the device.

In another series of experiments of this group, instead of discharging air into the altitude chamber, we introduced oxygen up to an overpressure of 2 atm for a period of time no greater than 5 min. This period of time is due to the fact that the dog's protracted stay in an oxygen atmosphere leads to a drop in the oxygen pressure in the tissues of the revived organism and to a

stoppage or worsening of breathing and cardiac activity (if they have already been restored).

Some of the thusly revived dogs that had succumbed to acute decompression and been in a state of clinical death for 5-6 min survived for a long time, and as early as the day following reanimation their behavior could scarcely be distinguished from that of normal animals.

The second method we developed for reanimating dogs that died from acute decompression differs from the first only in that during reanimation of the dogs neither artificial respiration nor massage of the heart is employed. After the pressure has been brought back to normal, air from a cylinder is pumped into the altitude chamber up to an overpressure of 0.5, 1.0, 1.5 and 2.0 atm and is then gradually let out and in its place oxygen is introduced up to an overpressure of 2 atm. Gradualy drawing off of the air leads to appearance of the ECG. Subsequently, the oxygen is first let out of the altitude chamber down to an overpressure of 1 atm and is then pumped back into the chamber up to an overpressure of 2 atm; these cycles succeed one another several times a minute. It turned out that this kind of action on the reviving organism leads to restoration of heart action and respiration and then, in a number of cases, to complete resuscitation of the dogs.

Let us quote a passage from the record sheet of one experiment of this type, which was conducted on June 10, 1971.

The dog was a female named Lya weighing 5000 g. Morphine-nembutal narcosis. The dog was placed in the altitude chamber, immediately whereafter (at 10 hours 45 min) the vacuum pump was turned on:

[illegible]	Altitude, km
10 hours 45 min 30 sec	8
10 " 46 " 00 "	15
10 " 46 " 20 "	20 (last inspiration)
10 " 46 " 30 "	22
10 " 49 " 20 "	22

Drawing off: 30 sec.

10 hours 49 min 50 sec: normal atmospheric pressure.

No respiration; ECG was a straight line.

Air from a cylinder was let into the chamber:

						Overpressure, atm
10	hours	50	min	30	sec	0.5
10	"	51	"	00	"	1.0
10	"	51	"	30	"	1.5
10	"	52	"	00	"	2.0

The air was replaced by oxygen. As the air was being drawn off, an ECG recording appeared.

10 hours 55 min 20 sec: oxygen began to be introduced into the altitude chamber. The ECG showed a considerable worsening of cardiac activity.

The oxygen overpressure in the altitude chamber was gradually brought up to 2 atm. There was no natural respiration. Then the oxygen was drawn off and its overpressure in the altitude chamber was again brought up to 2 atm. After two such "inspiration" and "expiration" cycles, at 10 hours 57 min 17 sec, the dog evinced its first independent inspiration, and soon the ECG recording was also resumed.

At 11 hours the dog was removed from the altitude chamber; it was sleeping (narcosis sleep); its respiration was deep, and of the diaphragmatic type: 7-8 times per min; all ocular reflexes were lively.

Thus, the dog was in the altitude chamber with a rarefied atmosphere for 4 min, of which 3 min were spent at an altitude of 20-22 km. The oxygen overpressure in the altitude chamber was brought up to 2 atm for the first time 9 min after the last inspiration. The dog's natural respiration was absent 10 min 57 sec. /8

19 hours: the dog's condition was unchanged.

21 hours: the dog raised its head and attempted to extricate itself from the bedding. No stool or urination as yet.

The following morning, the dog walked with sufficient assurance and reacted normally to its surroundings. Its condition is still good up to the present time (the end of March 1972): no complications whatsoever of the nervous system or other vital organs.

According to data in the literature, even a 2-min stay in an altitude chamber under a pressure of 40-30 mm Hg leads to the death of all experimental dogs.

The studies that go to make up the present report are especially heuristic in character, being devoted only to the problem of reanimation incident to clinical death due to decompression.

In this area, intensive further study will be required, but our preliminary experiments already indicate that the possibility of reanimation with the aid of the artificial blood circulation method or other new methods incident to such death is considerably greater than might have been expected. We have reason to suspect that the tank method of reanimation we have been describing -- without using artificial respiration or massage of the heart -- can also find an application in obstetrical practice, for the purpose of reanimating neonates that are perishing at the time of birth to the point of asphyxia.

Abstract

It has been considered that the stay of dogs for more than 2 min in an altitude chamber at an atmospheric pressure of 40-30 mm Hg always leads to their death. In our experiments, decompression was performed at a rate of 45-180 sec to a rarefaction of 30-18 mm Hg, and in this atmosphere the dogs remained no less than 1.5-3 min. Of the 34 dogs of the first group, three dogs, which had been in a state of clinical death for 10-18 min, after reanimation with the aid of the artificial blood circulation method survived for a long time with restoration of all their vital functions. In the second group of dogs, for the reanimation of which the tank method was used, better results were obtained, but only in those cases where clinical death lasted no more than 9 min. Five references, one table.

RESISTANCE OF SOME SPECIES OF COLD-BLOODED AND WARM-BLOODED ANIMALS TO EXPLOSIVE AND SLOW DECOMPRESSION

V. Ya. Lukhanin

Besides oxygen starvation, a rapid reduction in atmospheric pressure causes in animals a whole series of decompression phenomena [1-6, 11, 13-15]. In addition to considerably impaired physiological functions, some authors have noted certain morphological changes in the organism of animals subjected to the action of decompression. Thus, O. I. Parfenova [12] detected acute pulmonary hyperemia as a result of a sudden drop in barometric pressure down to 100 mm Hg and lower. Latner [27] has pointed to the genesis of regions of extravasation in the lungs, pericardium, liver and other organs of mice under the action of rapid decompression. Smith [28] detected extravasations and hemorrhages in rabbits and dogs subjected to decompression from 2400 to 13,500 m (564-110 mm Hg) for 0.02 sec. Whitehorn, Lein, and Edelmann [29] Edelmann, Whitehorn, Lein and Hitchcock [22] also noted extravasations in the lungs, heart and under the dura mater of dogs subjected to decompression from 523 to 87 mm Hg. Corey [20] has stressed the fact that at the time of autopsy extravasations into the pulmonary tissue are always detected. Cole [19] noted atelectases and hemorrhagic and emphysematous regions under the action of explosive decompression. Grognot and Senelar [25] have pointed to the presence of small macular subpleural extravasations in the lungs after explosive decompression. /8

Anatomicopathologic studies of the tissues of dogs subjected to the action of rapid decompression down to 2 mm Hg and lower was carried out by Dunn, Bancroft, Haymaker and Foft [21]. Most significant was the absence of noticeable pathologic changes in the liver, kidneys, brain, spleen and gastrointestinal tract of the animals, if the time of exposure to the final pressure did not exceed 120 sec. Under the action of more protracted exposure, stagnation phenomena arose in these organs. In the lungs, too, intraalveolar edema, subpleural extravasations, focal atelectases and peripherally arranged emphysematous changes have been detected. /9

The adduced data give an inkling of the nature of the morphological changes that may arise in animals under the action of decompression. The present study is devoted to elucidation of which morphologic disturbances are due to explosive decompression and which are caused by hypoxia and low pressure during the animals' subsequent sojourn under the conditions of the final, low barometric pressure. In our experiments, we used animals situated on different rungs of the evolutionary ladder and possessing different degrees of resistance to hypoxia.

Research method. In the present report we describe data from macromorphologic and partially histologic investigations of the organs of animals subjected to the action of slow or explosive decompression with subsequent exposure to low pressure. As cold-blooded subjects we used frogs (Rana esculenta), and as warm-blooded subjects, white laboratory mice and white rats coming from the line of the A. A. Bogomolets Institute of Physiology. In all, we investigated 124 animals.

Slow decompression was effected by evacuating the air from a 0.01-m^3 altitude chamber down to 10 mm Hg (as well as down to different intermediate pressures: 100, 80, 60, 50, 40 and 20 mm Hg) for 1 min, and explosive decompression, by suddenly joining the small chamber containing the animals to the large chamber in which rarefaction had been set up beforehand. The leakage factor amounted to 2.5 m^2/m^3, and the pressure ratio was 16, 35, 70. The pressure was determined with the aid of a mercury manometer.

Research results. The pulmonary tissue of frogs, explosive decompression from 760 to 50, 20 mm Hg did not cause any visible morphologic changes. The color of the lungs remained pale rose; no extravasations were noted. In animals that survived a 60-min exposure to a steady pressure after explosive decompression from 760 to 50 mm Hg, no changes were detected in the lungs. The lungs of animals that died at the end of a 150-min exposure had a greyish tint, but no foci of extravasation, atelectases or emphysemas could be detected. Nor did 30-, 60- or 90-min exposure after explosive decompression down to 20 mm Hg cause any macromorphologic changes in the lungs.

In connection with the formation of the "atmoperitoneum" (a phenomenon connected with the "boiling" of the peritoneal fluid), the organs of the abdominal cavity, especially the stomach, were driven back into the thoracic region, often with penetration into the thoracic cavity, and sometimes even with the emergence of the stomach into the oral cavity. This allowed the engorged vessels of the stomach wall to be observed. No gas bubbles could be detected in the vessels. Nor were they detected in the stomach when it was opened in water. If the stomach was not everted, autopsy disclosed that both the stomach and the intestine overflowed with gases. Detected behind the abdomen, under the fascia of the muscles, between the muscles and under the skin were small amounts of gas (vapor) left over from the interstitial emphysema after recompression. Here, minute extravasations were often detected which, however, had no significant effect on the animals' survivability.

The lungs of frogs subjected to the action of slow decompression down to 50, 20 and 10 mm Hg without exposure or with exposure lasting from 30 min to several hours, differed but little from those described above. The high resistance of frogs to hypoxia,

as well as the absence of marked damage to the lungs, facilitates spontaneous resumption of their breathing after recompression provided the heart keeps on beating.

In warm-blooded animals, the morphologic changes are more pronounced. The mice began dying after slow decompression down to 100 mm Hg lasting 30 sec and fast recompression, in spite of the /10
fact that only a small part of their lungs was put out of service. After slow decompression down to 80 mm Hg for 3¼ sec and fast recompression, the morphologic picture did not change. A 10-sec exposure after slow decompression from 100 or 80 mm Hg, though it did aggravate the changes in the lungs, did so insignificantly. Only in some of the animals did hemorrhagic atelectases encompass all of the lungs. In other experiments, however, in spite of a further drop in the final pressure, as well as exposure of the animals to low pressure, i.e., a longer duration of their stay under the conditions of anoxia, the amount of extravasations and atelectases did not increase, which is connected with their more rapid death and, consequently, a shorter period of agony.

After explosive decompression down to 50 mm Hg and fast recompression, no appreciable damage to the lungs could be observed. The animals survived. Death did not set in even after explosive decompression down to 20 or 10 mm Hg, though the morphologic picture in the lungs did worsen somewhat. As a rule, if the duration of exposure after explosive decompression down to 50 mm Hg did not exceed 5 sec, pronounced damage to the lungs was absent. An increase in the duration of exposure to 10 sec and more led to an increase in the amount of extravasations and atelectases. But complete consolidation of the lungs was seldom to be observed.

As in the case of the preceding series of experiments, explosive decompression down to 40, 20 or 10 mm Hg without exposure or with a 5-sec exposure caused no significant damage. As a rule, a 10-sec exposure caused more pronounced damage and led to the death of the animals. The presence of general interstitial emphysema did not change the morphologic picture in the lungs and exerted no effect on survivability. Pronounced "boiling" of the tissue fluids did not develop until the animals' death, while almost imperceptible emphysemas developed somewhat earlier. This, however, did not aggravate the situation, and the animals' death generally set in after the same intervals of time as in the case of the experiments that were not accompanied by the development of interstitial emphysema.

The rats turned out to be more resistant to our experiments than were the mice. In the lungs of animals subjected to slow decompression from 760 to 100 mm Hg for 30 sec and fast recompression, only an insignificant amount of extravasations could be observed, which had no visible harmful effect. Microstudies showed that only small areas of the pulmonary tissue were affected by extravasations spread by capillaries compressing the lumen of

the alveoli (Fig. 1). Small areas were filled with edematous liquid. In places in the fine bronchi and bronchioli, an accumulation of cast-off epithelial cells was noted. In most of the pulmonary tissue, sections of relatively normal tissue alternated with sections with moderately dilated and engorged capillaries of the interalveolar septa.

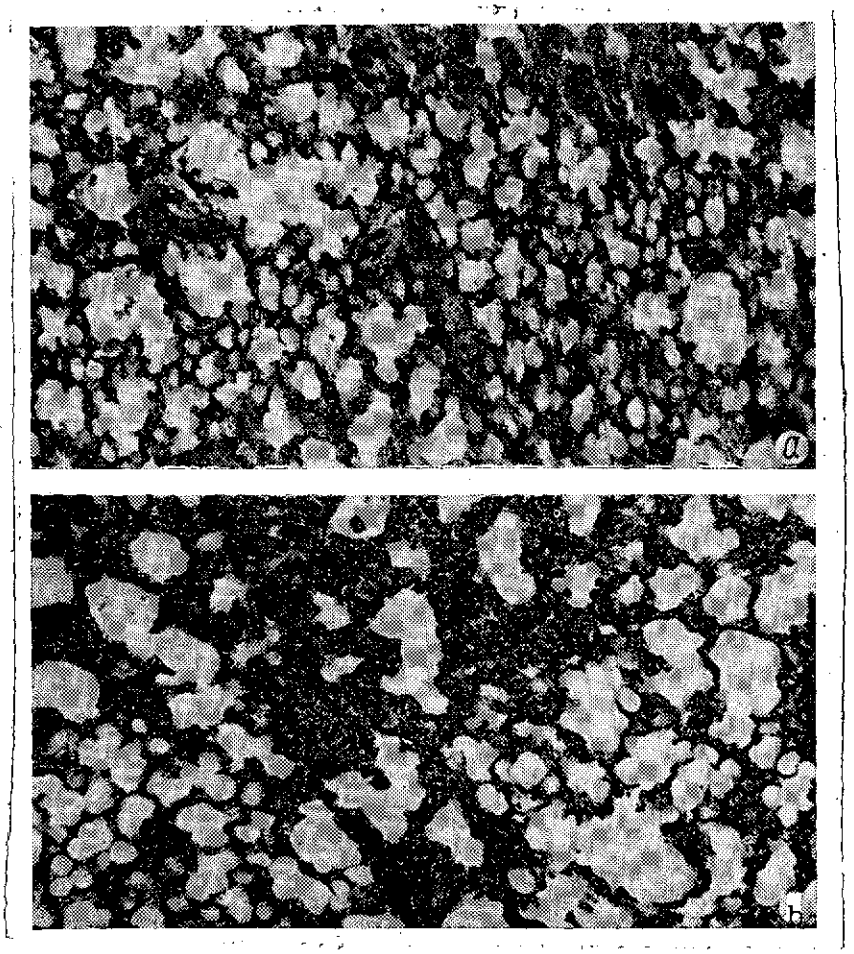

Fig. 1. Pulmonary tissue of rat (250x). a. Normal; b. After slow decompression down to 100 mm Hg.

No significant differences could be observed in the lungs of rats subjected to decompression down to 80 mm Hg for 34 sec and fast recompression. The picture changed sharply if, after decompression down to 100 or 80 mm Hg, the mouse was kept at the final pressure for 10 sec. This time sufficed for extensive hemorrhagic atelectases embracing all lobes of the lungs to develop. The lungs turned a dark cherry red. Microscopic examination

revealed that much of the pulmonary tissue was occupied by regions in which blood-filled alveoli alternate with alveoli in which extremely dilated capillaries are engorged and compress the lumen. The areas of extravasations into the cavity of the alveoli were immense (Fig. 2), and in the inconsiderable part of the alveoli that were preserved, a pronounced stagnant hyperemia of the capillaries of the alveolar septa could be observed. In those regions where the alveoli were filled with erythrocytes, there were foci of edematous fluid. Most of the pulmonary vessels were also engorged. In the lumen of many of the fine and medium bronchi containing edematous fluid, a considerable amount of cast-off epithelial cells was noted. /11

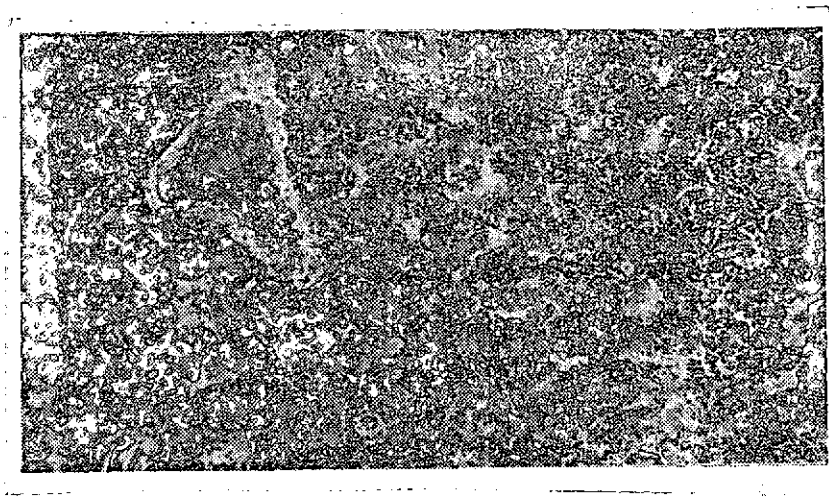

Fig. 2. Changes in the pulmonary tissue under the action of slow decompression down to 100 mm Hg and 10-sec exposure to final pressure (250x).

Similar changes could be observed in the lungs of animals subjected to slow decompression down to 60 (for 35 sec) and 50 (for 39 sec) mm Hg with fast recompression. In these experiments, the animals died without any additional exposure to the indicated pressures. In the experiments with slow decompression down to 20 mm Hg for 57 sec and fast recompression or with exposure to the final pressure for 30 sec, complete consolidation of the lungs with blood set in. In these experiments, the percentage of animals with lungs that were not completely shut off from respiration dropped to zero.

Explosive decompression down to 50 mm Hg accompanied by fast recompression led to formation of a certain amount of extravasation, but this had no effect on the rats' survivability. Not even difficult breathing was noted. But if, after explosive decompression,

the animals were kept at the final pressure for 20 sec, the lungs developed hemorrhagic atelectases that embraced a smaller or greater amount of the lobes, which led to a high death rate. Incident to exposure lasting 30 sec or longer, the lungs became completely hepatized and they were excluded from the act of respiration. All the animals perished.

The picture of the lungs of rats subjected to explosive decompression down to 200 mm Hg and fast recompression was the same as after explosive decompression down to 50 mm Hg. After explosive decompression down to 20 mm Hg and a 15-sec exposure, the animals survived; but they showed signs of very difficult breathing. Autopsy disclosed that a considerable part of the lungs was filled with extravasations and atelectases. A 30-sec exposure led to formation of a hemorrhagic atelectasis that was either extensive or encompassed all of the lungs. With more protracted exposure, the indicated changes became maximal. The onset of decompression interstitial emphysema in the animals of this series of experiments did not lead to any apparent additional morphologic damage to the lungs as compared with the animals of the preceding series of experiments. In many of the dead animals, hemothorax was detected. /12

Discussion of experimental results. We have established that, in contrast to what was reported by Cole et al. [19], explosive decompression does not cause significant morphologic changes in the lungs. Nor do our data agree with the reports of Gell, Hall and Mostofi [23] that the death of the animals (rats) is due to an increase in the volume of gases pervading the organism at the time of explosive decompression, and not to anoxic anoxia. The authors of Ref. [21] used an original method: after explosive decompression, these authors, without returning to the initial pressure, fixed the animals in formalin under rarefied atmospheric conditions. Recompression was performed after the tissues had been completely fixed and the changes had taken place. But inasmuch as recompression was not fast, the authors should not have asserted that death set in instantaneously and that the animals died of explosive decompression. After explosive decompression, before being fixed in formalin, the experimental animals they employed were subjected to the action of a low final pressure. In the interval between the explosive decompression and the animals' fixation, in their organism the symptoms of decompression interstitial emphysema cropped up. Inasmuch as recompression did not take place, the emphysematous symptoms did not disappear and were fixed. The huge cavities that autopsy disclosed in the subcutaneous cellular tissue and in the peritoneal and thoracic cavities give the impression of total destruction of the organism by the spreading vapor of "boiling" tissue fluids and by spreading gases.

The data we obtained for less highly-organized animals [7-10], as well as the data we have presented above, indicate that the spreading of the interstitial and intracavitary gases and vapor /13

from tissues fluids is not an important traumatizing factor. After explosive decompression as well as after slow decompression, in spite of protracted exposure to low pressure, at which time emphysematous symptoms appear, the frogs survived, if exposure time did not exceed the lethal length. In spite of the appearance of noticeable emphysematous symptoms, incident to unprolonged exposure, the rats and mice survived, too.

The duration of survival also remained the same in those experiments in which the final pressure was rather high and decompression interstitial emphysema did not arise. Evidently, therefore, in the given case it is possible to speak of emphysematous symptoms only as an aggravating factor. This was pointed out as early as 1957 by A. G. Kuznetsov, who conducted a detailed analysis of the process of "boiling" of tissue fluids at high "altitudes" and suggested calling the symptom that develops in this case "decompression interstitial emphysema."

The mortal danger of explosive decompression for animals has been denied by Hall and Corey [26]. They noted hemorrhages with rupture of the alveoli in the lungs of rats and pointed to the relationship between the extent of such damage and the duration of the animals' stay under reduced pressure. Gelfan et al. [24] also think that the degree of pulmonary damage depends for the most part on the duration of the animals' exposure to the final pressure, without denying that explosive decompression may facilitate the genesis of such damage. Ye. A. Kovalenko et a. [3] have pointed to the considerable and acute disoxygenation of the tissues of animals at high "altitudes."

Nor did we ever detect extensive damage of the lungs after explosive decompression. Apparently it arises as a result of the spasmodic contractions of the chest that can be observed during exposure to low pressure (hypoxia), after explosive or during slow decompression. After more prolonged agony, the changes are more considerable. Mice die from hypoxia faster; therefore, the concomitant greater damage cannot be so frequently observed. In frogs, such damage was not detected at all -- apparently because of the absence of spasmodic respiratory movements. The rougher structure of the pulmonary tissue of frogs is also a contributory factor.

Thus, the main cause of death of the experimental animals and of the morphologic changes in their lungs is the hypoxia (anoxia) to which the animals are subjected during postdecompression exposure to a low final pressure and during slow decompression.

In experiments with explosive decompression and subsequent rapid recompression to the initial pressure, this factor had practically no effect because of the brevity of the entire process. As we have just said, the mice died faster than the rats, and the

morphologic changes in their lungs were less pronounced, which must be connected with the lower resistance of mice to hypoxia [14]-17], the more rapid consumption and desaturation of oxygen together with other gases from the animal's organism [3], as well as the smaller oxygen reserve in their organisms.

The longer agony of the rats, which is due to their somewhat greater resistance to hypoxia, leads to formation of more extensive extravasations in their lungs. Incident to unpronounced changes in the lungs and briefer exposure, the animals could survive; but incident to considerable damage, the absence of respiratory capacity prevented resumption of normal breathing and the animals' survival. In the lungs of frogs, which are less highly-organized animals with a high resistance to hypoxia [15-17], significant damage could not be detected. After explosive as well as slow decompression, they survived even after a rather prolonged stay (for several hours) under conditions of extremely low barometric pressure (10 mm Hg).

I. M. Khazen [18] has reported on the death of mice and rats under the action of sudden decompression, as well as on the greater resistance of the more highly-organized animals to this factor. /14

Abstract

It is noted that under the action of explosive decompression accompanied by fast recompression, animals do not die, and the morphologic changes are less considerable than in the case of slow decompression. The presence of more pronounced lesions in the lungs of warm-blooded animals, as well as their death under the action of slow decompression, is connected with the effect of anoxia. Twenty-nine references, two figures.

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EFFECT OF COMPLEX PREPARATIONS OF PHEPLAVINE AND PLAVEPHINE ON ANIMALS UNDER ACUTE HYPOXIA AND HIGH-MOUNTAIN CONDITIONS

A. V. Shapovalov

Of the large group of pharmacologic substances possessing a more or less pronounced antihypoxic effect [4, 5, 12], the attention of researchers is being particularly attracted to those neurotrophic substances that enhance the functions of the physiologic compensatory mechanisms of the human and animal organism [2, 18], which opens up the possibility of using them in space flights [21]. /14

In our experiments, we studied the effect of complex preparations of Pheplavine (PhPKB mixture) and Plavephine (PPKB mixture), which are used for prevention of "air" and "sea" sickness [17], on the resistance of animals to acute hypoxic hypoxia and limiting physical loads under lowland and high-mountain conditions. /15

Method. The PhPKB mixture consists of phenamine and platyphilline bitartrate (0.005 g), papaverine hydrochloride (0.05 g), pure caffeine (0.1 g) and potassium bromide (0.15 g). The PPKB mixture differed from the PhPKB mixture only in containing no phenamine.

The pheplavine was administered subcutaneously to male white mice and rats of the Wistar line in aqueous solutions 1-1.5 hours before the start of the experiment, estimating the optimal dose of phenamine to be 0.0002 g/kg for mice and 0.0001 g/kg for rats [7]; the plavephine was administered in exactly the same dose as the pheplavine. Corresponding doses of physiologic fluid were administered to the control animals.

The state of acute hypoxia was attained by "elevating" the animals in an altitude chamber at a constant rate of 10 m/sec to an "altitude" of 10 km. This was followed by "halt" and "descent". To test the animals' endurance to limiting physical loads, we used swimming with a load (10% of body weight). In all, we used 220 mice and 78 rats in our experiments. The results were statistically processed [14, 15].

Results and discussion. In both series of experiments involving "elevation" of white mice in an altitude chamber to an "altitude" of 10,000 m, the animals' survivability amounted to 50 ± 7.9% in both experimental groups (PhPKB and PPKB) as against 30 ± 7.2% in the control group (for 30-min exposure). For 2-hour exposure, the survivability after administration of the PhPKB mixture amounted to 25 ± 9.6%, while the PPKB mixture increased survivability up to 35 ± 10.6% as against 20 ± 8.9% in the control.

In the case of the white rats, we investigated the effect of pheplavine (PhPKB) on the animals' resistance to limiting physiological loads. We set up three series of experiments: under lowland conditions (Kiev) and high-mountain conditions (settlement of Terskol, Kabardino-Balkarskoy ASSR, 2200 m) before and after preliminary acclimatization.

In the first series (Kiev), the pheplavine increased the mean swimming time (M) of the experimental group by 11.9 min as compared with the control. In the second series (Terskol, fifth day of the animals' stay), this time was still less than in the control (12.7 ± 1.03 and 13.0 ± 1.0 min, respectively). The variations in this series were not statistically significant. In the third series (Terskol, 35th day), in the animals acclimatized for 30 days to an altitude of 3300 m, the mean swimming time in the experimental group increased by a factor of almost 3 (48.8 ± 17.03 min) as compared with the control (16.2 ± 4.4 min). In all three series of experiments, the variability in the duration of swimming was considerably higher in the experimental groups than in the controls. Apparently, the reactions of the animal organism to administration of phenamine depends mainly on the state of the CNS, the distinctive features of whose response to administration of the preparation may in turn change the physiologic "chances" of developing adaptive reactions [3, 9, 16].

The rectal temperature of the unfixed rats 1 hour 30 min after administration of the PhPKB was reliably increased by 1.1° only in the first series ($P < 0.01$). For the same period of time, the oxygen pressure (pO_2) of the control and experimental groups, which was determined by the polarographic method (recording from five points) in the muscles of the tail ends (or the pO_2 of the physiological solution), was on an average equal to 21.6 and 19.2 mm Hg, respectively.

Our data to a certain extent confirm the data in the literature about the possibility of increasing the organism's resistance to the action of extremal factors by pharmacologic means. Incident to short-lived hypoxia, phenamine (PhPKB), by acting on the adrenergic systems of the reticular formation of the brain [8, 11] during formation of the adaptotrophic and at the start of the compensatory phases, in the first 30 min from the moment of onset of the stress situation, when the functional activity of the adrenergic systems predominates [10], may facilitate more rapid mobilization of adaptive mechanisms that are advantageous to the organism under these conditions [13, 22, 23]. This may also be facilitated by a change in inhibitory cortico- and thalamofugal effects (phenamine, caffeine, bromides) [17]. Incident to protracted hypoxia, simulation of the adrenergic system leads to reduced altitude resistance [1, 6, 20]. Under these conditions, it is obvious that it is more expedient to use the PhPKB mixture (papaverine, platyphilline), which weakens interoceptive

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effects from the internal organs which, under hypoxia conditions, may be of a pathologic nature [19].

Thus, pheplavine and plavephine increase animal resistance to hypoxic hypoxia (PhPKB and PPKB mixtures) and to limiting physiologic loads after acclimatization to high-mountain conditions (PhPKB), which holds out prospects for further study of the action of these preparations under hypoxia conditions.

Abstract

In experiments with male white mice and rats of the Wistar line, the possibility was investigated of using complex preparations of pheplavine and plavephine to enhance the resistance of the animals to acute hypoxia incident to "ascent" in an altitude chamber and under high-mountain conditions. It is shown that subcutaneous introduction of the investigated preparations 1-1.5 hours before the start of the experiment was conducive to an increase in the resistance of the animals to extremal effects. Looked into are the considerable individual differences in the response of acclimatized animals to the introduction of pheplavine. Twenty-three references.

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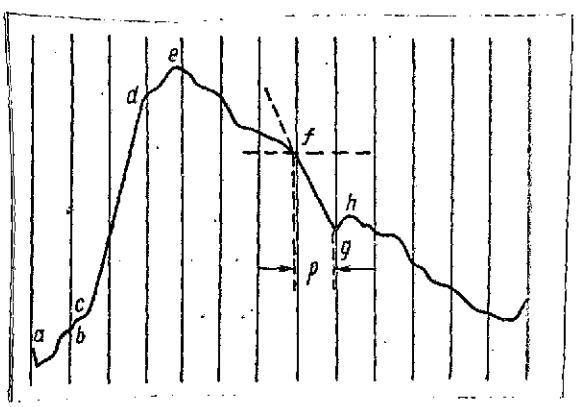
ON THE EFFECT OF GRAVITATION ON THE HUMAN ORGANISM

V. L. Zil'man

Gravitation is one of the most powerful factors determining /16
the biological status of living organisms. This becomes all the clearer, the more the attention of researchers turns to cases of changes in the customary action of gravitation and their consequences for living systems. Korzhuyev [3], for example, considers that an evolutionary event of so great a significance as the transition of aquatic animals to a terrestrial form of life had, among others, the significance that instead of conditions of relative weightlessness in water, the reptiles of the Paleozoic era found themselves under conditions of full weight at the bottom of an ocean of air, which for them amounted to a peculiar hyperweight. Since that time, for the majority of terrestrial animals, the action of gravitation has been stable, if we leave out of consideration daily gravitational perturbations of short duration, which are connected with natural locomotion.

Man also experiences these perturbations; in addition, he is subjected to rather considerable, albeit short-lived, actions and accelerations connected with his utilization of technological means of transportation. But, in contrast to the majority of other surface dwellers, man in his development lived through still another (phylogenetically speaking, second) "gravitational revolution" due to the transition of his prehistoric ancestor to a vertical posture. At the present time he finds himself on the threshold of a third revolution that is connected with his emergence into space. In the latter case, we have in mind not so much the g-loads and the state of weightlessness that arise during space flight as we do the uncustomary gravitational conditions man will encounter -- and is already encountering -- on extraterrestiral bodies comparable in mass to Earth. In this connection, a whole series of problems arises that deserve scientific consideration and investigation.



Sphygmogram of the carotid artery: cd -- anacrotic upstroke, p -- protodiastole. Scale division: 0.05 sec.

Full-scale artificial simulation of the above-mentioned conditions is evidently unrealistic, but some of their elements can be imitated. Thus, a man's remaining in an upright position for a long time (and even for a short time) -- after bedrest, hypokinesia or an intensive muscular load) to a certain extent reproduces the conditions of increased gravitation. The decompensation of the antigravitational mechanisms of the cardiovascular system in the form of gravitational shock, "syncope on the hour" etc. can also be quite legitimately regarded as a consequence of the action of a supernormal force of gravity.

Bringing a man's body into a horizontal position and a position with the head down (antiorthostatism) approximately imitates the physiological conditions of a gravitation that is reduced as compared with Earth's. In the limiting case of antiorthostatism (180° relative to the body's normal vertical position), the hydrostatic blood pressures in the regions "heart - feet" and "heart - head" has an opposite direction; by varying the head's comparatively small angles of inclination up and down, we can achieve different degrees of reduction in the normal hydrostatic pressure in the region "above the heart" and an increase in it in the region "below the heart."

In our experiments with 98 examinees on a rotating testbed, we set up, in particular, an antiorthostatic position (150° relative to the body's orthostatic position) for 1.5-2 min. Our attention was attracted to the changes in the protodiastolic period (protodiastole) -- the time from the start of the return flow of blood in the aorta to the closing of the semilunar valves, which can be measured on the sphygmogram of the carotid artery (see figure).

According to certain data [2], the duration of the protodiastole varies within the limits 0.03-0.04 sec, but, according to our data (incident to the body's horizontal position), it varies within the limits 0.02-0.06 sec. As a rule, incident to transition from the horizontal position to a position with the head down, /18 the duration of the protodiastole decreases on the average by 0.02-0.03 sec. Being unable to find the results of other authors in the literature available to us, we cannot compare them with our own; but there are indications [2, 9] that the protodiastole shortens incident to retarded outflow of blood from the heart, as well as with increasing arterial resistance. The latter can be excluded from our experiments with antiorthostatism inasmuch as we regularly noted a reduction in the general and specific peripheral resistance.

Thus, the cause of the described changes in the protodiastole may be a retardation of the outflow of blood from the heart that is due to a profound change in the hydrostatics of the bloodstream in connection with the body's antiorthostatic position. It is

obvious that, in spite of the reduced resistance of friction, to overcome the hydrostatic resistance in the region "heart - feet" below the heart requires a more forced action of the heart than to overcome this resistance in the region "heart - head" above the heart, i.e., incident to orthostatics. This fact bears witness to the profound physiological conditionality of the height of the heart's position in the human body, namely, near the brain.

Returning to the above-mentioned phylogenetic shift caused by the transition of man's remote ancestors to an upright position and an erect gait, it should be noted that what has been most frequently studied is changes in the support-motor apparatus. The effect of the body's vertical position on other, in particular, visceral, systems has been less thoroughly studied.

In assuming an orthostatic posture, our remote ancestors so reoriented his body that its long axis with two poles (head and lower extremities) coincided with the direction of the lines of force of the gravitational field. Already in the studies of O. Hertwig [8] it was shown that even such an intimate process as the cleavage of the ovum depends on the orientation of its poles in the gravitational field. It cannot be conceived that the visceral systems of the human organism remained indifferent to such a change. In this connection, for example, it has been supposed [1] that man's appendix was transformed into an organ that regulates the downward motion of the chyme along the vertical sections of the intestine.

It is probable that the cardiovascular system of man also adapted itself to his vertical posture. There really is a gravitational redistribution of the vascular tone [4], but at the same time we can expect that such a stably acting factor as the usual direction of the gradient of gravitation relative to man's body must be attached to stably represented morphologic peculiarities of his cardiovascular system, and all the more so since such peculiarities have been found in those animals which, like man, have "special relations" with gravitation. Thus, in giraffes [6, 7, 10] have been found, in particular, valves in the arterial vessels of the neck, a very insignificant elasticity of the vascular walls here, etc. Moreover, animals for which a position with a lower head is characteristic (sheep, cows) have a particularly well developed "rete mirabile" in the form of an extensively ramified system of arterial vessels along which the blood is supplied to the skull [5].

The functional and morphologic pecularities of man's cardiovascular system that are connected with its adaptation to the gravitational polarization of his body can be most graphically shown by comparison with such peculiarities in an animal with the opposite polarization. Such an animal -- perhaps the only one -- whose normal position most of its life is antiorthostatic, is the bat.

In the course of our investigation, we immediately measured in the animals and in human corpses the length of the thoracic and abdominal sections of the aorta (l_1) to the bifurcation and the length of the left common carotid artery (l_2) to the bifurcation, regarding these two vessels as a single vascular basin subject to more or less the same hydrostatic conditions. The height of the heart was calculated as the percentage ratio of the upper section of the trunk (artery) to the lower (aorta), i.e., as l_1/l_2. It was assumed that in connection with the natural anti-orthostatic position of the bat's body, its heart ought to be considerably more distant from the head than in other animals of the same class that are close to it in size and general body form. /19

Our assumption was confirmed, as can be seen from the data below

Man	430.0	119.5	27.8
White mouse	46.0	12.0	28.0
Rabbit	286.0	87.0	30.4
Pig	730.0	360.0	49.3
Bat	34.0	17.5	51.5

Noteworthy is the especially obvious difference between the index l' in man and pig -- an animal which is comparable in size to man, and which, as is known, has much in common with him as regards the structure of its internal organs and cardiovascular system.

Thus, the gravitational polarization of man's body in Earth's gravitational field leads to distinct and stable morphofunctional changes in his cardiovascular system. These changes may, in general, depend not only on fluctuations in the direction of the gravitational gradient, but also on its absolute magnitude. It is necessary to point to the greater variability of the index l' in man than in animals. This gives a reason to suppose that individual differences in resistance to g-loads, in orthostatic resistance, etc. in man definitely depends on the height of the heart in the arterial-aortic trunk, from which ensues the necessity of taking into account the given index in the specialized selection of people.

Abstract

The authors investigate the sanguiferous system of man and some animals: bats and white mice, rabbits, pigs. It is shown that the location of the heart on the aortic-arterial trunk is connected with peculiarities of the organism's ecology. The normal orthostatic position of man's body causes a high position

of the heart, which apparently ensures the brain a sufficiently reliable blood supply. For the same reason the heart of the bat, the usual position of whose body is antiorthostatic, is shifted in the caudal direction. The data obtained complement theoretical concepts about the paths of evolution of man's cardiovascular system, including the path connected with man's emergence into outer space, and some correctives are introduced into the methods of specialized selection of people. Eleven references, one figure.

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STATE OF CUTANEOUS RECEPTION INCIDENT TO LIMITATION OF THE ACTION OF SOME FACTORS OF THE EXTERNAL ENVIRONMENT AND HYPODYNAMIA

A. K. Podshibyakin, V. A. Tarasenko, and G. F. Tkach

In theoretical and practical studies, ever more attention is being devoted to the biologic rhythms, the peculiar "biologic clock" of living organisms, the functioning of which is synchronized with helio-geophysical cycles. Of particular interest is elucidation of the changes in the organism's physiologic parameters incident to weakening of the usual (customary) rhythm of action of some factors of the external environment. /19 /20

As early as 1926, Academician A.A. Bogomolets [2] pointed out that, without becoming pathologic, no rhythmic process in the organism can infringe on the law of its rhythm. It is important to note the remarks of I. R. Tarkahnov [5] and V. V. Pashutin [4] to the effect that in the process of isolating a man from the action of factors of the external environment, his sensing of cold, heat and other physical agents becomes intensified.

At the present time, in the manned compartments of spacecraft, the temperature is maintained at 17-23°C [1], which is optimal for the astronaut's organism, as well as for the performance of measuring devices. Spacecraft lack the natural succession of day and night and the concomitant temperature variation to which astronauts living under terrestrial conditions are accustomed [3]. Moreover, under space conditions the muscular loads on man are considerably reduced. Hypokinesia can therefore be regarded as one of the elements that under these circumstances accompany limitation of the action of the above-mentioned physical agents. /21

The purpose of our study was to investigate the reactions of the skin to thermal, tactile and electrical effects incident to a reduction in the customary daily rhythm of drops in temperature and mechanical effects.

Research method. In May-July 1970, six volunteer examinees hospitalized in a ward with a stable temperature were subjected to prolonged hypokinesia. During airing of the room, the investigated parts of the skin of the forearm and foot were covered with blankets, and their condition was not affected by the slight reduction in temperature due to fresh air entering the ward. Hypodynamia was not severe. The examinees were allowed to turn their body, move their hands while eating and perform the natural functions.

All measurements (tactile sensitivity, number of effective cold points and cutaneous sensitivity to an electric current) were carried out 1/2 hour after breakfast. Two-dimensional tactile sensitivity was determined by measuring the distance in mm between the jaws of a caliper on the skin of the forearm, and the number of effective cold points (on 1 cm^2 of skin of the forearm), by touching with a 1 mm^2 metal rod with a temperature of about 0°C. To study changes in cutaneous sensitivity to an electric current, an ESL-1 stimulator was used. The faradic excitability was measured every morning at one and the same point on the skin of the foot during the entire period of hypokinesia. Our criterion was the voltage at the onset of perception of the action of the electric current.

The investigations were carried out before and after cooling by 10° the skin of the examinee's foot, independently of its initial temperature. One foot was cooled by means of an ice-filled, rubber, hot-water bag. The changes in temperature of the skin of the foot were recorded by means of an GOS-M thermal resistor fastened to the arch of the foot, and a microammeter calibrated to the 10 to 37°C temperature range we studied.

Observation results. With a stable microclimate of the room, the two-dimensional tactile sensitivity of the skin of the forearm was intensified from 22 ± 0.07 mm (before limitations) to 17 ±0.4 mm at the end of observation; at the same time, incident to the test involving cooling of the foot, the difference in the indices between measurements (before and after the test) was increased from 4.4 ± 0.01 to 6.0 ± 0.01 mm.

Similar effects were detected when investigating the skin of the foot. In particular, it ensues from these observations that incident to restriction of thermal and mechanical effects on the receptory apparatus, the time of restoration of the temperature of the skin of the foot to its initial value after cooling of the foot, increases reliably ($P < 0.001$; see Table 1).

Incident to a deficit of external effects that is connected with an attenuation of the daily rhythm of action of temperature and mechanical agents, the number of effective cold points on 1 cm^2 of skin of the forearm increased from 10.8 ± 0.87 at the start of observations to 12.6 ± 1.36 at the end of restrictions, and incident to cooling of the foot, it dropped at the start of observations from 10.8 ± 0.87 to 7.8 ± 1.01 and at the end, from 12.6 ± 1.36 to 9.2 ± 0.97.

Incident to daily measurements of the faradic excitability of the receptory apparatus of the skin, the magnitude as well as the scatter of the values of the voltage of the electric current perceived by the examinees increased, depending on the period of restriction of outer effects. The fluctuations in voltage of the /22

TABLE 1. DISTINCTIVE FEATURES OF THE CHANGE IN THE TWO-DIMENSIONAL SENSITIVITY OF THE SKIN ON THE ARCH OF THE FOOT IN THE TIME OF RESTORATION OF ITS TEMPERATURE TO ITS INITIAL VALUE AFTER COOLING, BEFORE AND AFTER TERMINATION OF HYPOKINESIA (1970)

Examinee	Date of study	Temperature, °C			Sensitivity of skin on arch of foot, mm		Time of restoration of temperature of skin on arch of foot
		Room	Foot				
			Before cooling	After cooling	Before cooling	After cooling	
P-s'	14.V	21,0	28.6	18.5	22	26	6min30 sec
	16.V	22,0	30,0	19.5	30	39	7 » 03 »
	22.VI	24,0	28,0	18,0	14	20	11 » 45 »
	23.VI	24.0	34.0	24.0	8	25	12 » 25 »
K-r	15.V	21,0	30.0	20,0	12	21	10 » 53 »
	19.V	21,5	34,0	24.0	20	23	10 » 50 »
	24.VI	24,0	33,8	24,0	8	15	20 » 35 »
S-n	15.V	21.0	30.0	20,0	15	28	16 » 45 »
	19.V	22.0	25.0	15.0	19	20	13 » 20 »
	25.VI	24,5	30,5	20.5	4	17	25 » 43 »
Kh-y	17.VI	24,0	36.5	26,5	12	4	7 » 40 »
	30.VI	25,5	32,0	22.0	4	6	21 » 10 »
K-y	17.VI	24.0	28.0	18.0	18	8	13 » 43 »
	1.VII	24,5	32.3	23.1	8	11	20 » 29 »
T-n	18.VI	23.5	29,5	19.0	8	13	11 » 29 »
	30.VI	27.0	33.5	23.5	4	7	16 » 12 »

electric current that the examinee begins to perceive in the initial, intermediate and final periods of sensory and motor restrictions, estimated in coefficients of variation, increased; consequently, restriction of the action of temperature and mechanical agents of the external environment leads to an imbalance in the perception of electric stimulation by the cutaneous sensory system (Table 2). This imbalance, in our opinion, must be taken into account when using the cutaneous analyzer as a communication channel. Incident to a deficit of external stimulations, the magnitude of a signal in this type of communication, expressed in terms of the voltage of an electric current, must increase; otherwise, a man would be unable to perceive the information transmitted to him.

The change in the state of the cutaneous sensory system incident to restriction of the action of physical factors of the external environment may be connected, on the one hand, with a

TABLE 2. DISTINCTIVE FEATURES OF CHANGES IN THE FARADIC EXCITABILITY OF THE RECEPTORY APPARATUS OF THE SKIN (V, VOLTS), ITS VARIABILITY (C, %) IN THE PROCESS OF RESTRICTING TEMPERATURE AND MECHANICAL EFFECTS

Examinee	Dates of hypokinesia and limitation of action of factors of ext. environment	Observation periods									P_{AC}
		Initial			Intermediate			Final			
		n	V	C	n	V	C	n	V	C	
P-s'	25.V—22.VI	7	32.1±1.91	10,1±2.6	9	36.1±2.66	10.8±2.5	9	40.4±2,89	16.1±3.7	$<0,02$
K-r	26.V—25.VI	7	27.5±1.21	9.0±2.4	9	28.8±1.74	7.2±1.6	9	31.6±2.04	10.4±2.4	<0.05
S-n	26.V—25.VI	7	26.4±2.91	3.0±0.8	9	31.1±2.32	6,6±1,3	9	34,4±3,53	18.2±4.2	<0.05
T-n	21.VI—30.VI	5	35.0±0.0	0.0±0.0	—	—	—	5	37.0±3.37	28.1±8.7	<0.01
Kh-y	20.VI—29.VI	5	31.0±2,14	17,7±4.9	—	—	—	5	35,0±3.07	23.9±7.4	<0.01

peculiar atrophy (due to inactivity) of the perception of the electric current and, on the other, with an increase in the sensitivity of the receptory apparatus to stimulation, which is seldom encountered; i.e., with an absence or weakening of adaptation to its effects [4, 5].

In addition to these investigations, we studied changes in the frequency of the pulse, respiration and oxygen consumption in the examinees. If the pulse rate amounted to 67.4 ± 3.6 strokes/min at the start of hypokinesia, at the end of hypokinesia it increased to 82.8 ± 5.03. There was a corresponding increase in the breathing rate (from 17.7 ± 2.47 to 20.3 ± 3.17 breaths/min). The oxygen consumption increased somewhat (from 0.69 ± 0.48 at the start to 0.72 ± 0.12 ℓ/min at the end of hypokinesia). This increase appeared more marked in the tests involving cooling of the foot (from 0.13 ± 0.05 to 0.19 ± 0.05 ℓ/min).

Thus, the data we obtained suggest that it would be expedient to create in the cabins of spacecraft such a microclimate as would take into account the natural rhythmic drops in temperature that are encountered in man's daily life on Earth.

Abstract

In man, incident to hypokinesia and a restriction of the influence of daily temperature variations and mechanical effects, the threshold of perception of an electric current by the cutaneous receptor apparatus is increased, as are tactile sensitivity and the number of cold points, and the restoration time of the skin's local temperature after cooling is lengthened. These changes must be taken into account when using the cutaneous analyzer as a communication channel.

It might be wise to create in spacecraft cabins a microclimate with the daily temperature variations that man encounters under terrestrial conditions. Five references, two tables.

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BEHAVIOR OF FISH UNDER CONDITIONS OF SHORT-LIVED WEIGHTLESSNESS

V. Ya. Mamontov

To study the behavior of fish under conditions of short-lived weightlessness, our experiments were carried out in aquaria filled with water to different degrees against the background of the change in the state of water in weightlessness. This experimental setup was dictated by the available theoretical and experimental data on the state of a fluid under conditions of weightlessness [1,3,9,10], in which case in a quiescent fluid, only the forces of surface tension and the elastic forces of pressure from the walls of the vessel on the fluid, or the layers of the fluid that are contiguous to one another, can act. The state and shape of the fluid's surface are determined only by these forces. In a wetting fluid, such as water is for glass or (in our experiments) for organoglass, the surface of the fluid filling the vessel is, incident to normal gravitation, essentially plane, and at the interface of the fluid there is a scarcely discernible meniscus. /22 /23

Under conditions of weightlessness, the state of the fluid's surface is completely determined by the angle of contact at the phase interface, by the shape of the vessel and by the amount of fluid therein. The surface of the fluid becomes spherical. Also absent from the fluid in a state of weightlessness is the hydrostatic pressure, and a number of phenomena associated therewith disappear. The laws of communicating vessels and the law of Archimedes lose their force.

The experiments on the behavior of fish under conditions of short-lived (up to 30 sec) weightlessness were carried out aboard a TU-104A laboratory-aircraft while flying along a zero-G parabola (a Kepler parabola).

The experimental subjects were intact fish: tilapia (Tilapia mossambica Peters), silver carp (Carassius auratus gibelio Bloch), and common carp (Carassius carassius Linné).

The fish were placed in a container consisting of an aquarium with two 20-ℓ compartments and a movie camera, which was securely attached to the aquarium. Fastened to the sides of the aquarium was a bias lighting of eight lamps (28 V, 38 + 38 W), which gave a luminous flux of about 7680 lm (3840 lm per side). The movie camera had a 35-mm objective and a cassette with a 60-m-long 35-mm film. Filming took place at a rate of 24 frames/sec. One compartment of the aquarium was almost completely filled with water (compartment A). Only a small air blanket was left over. The second compartment was 1/5-1/10 filled with water (compartment B).

During the flight, for 3-5 sec up to the setting in of weightlessness conditions, the operator switched on the bias lighting and the movie camera qnd switched them off after the aircraft emerged from weightlessness conditions. In all, 68 experiments with short-lived (up to 25 sec) weightlessness were carried out.

The physical conditions in our experiments were similar to those described in the study of I. I. Kas'yan [4]. During execution of the parabolic trajectory, for 10-15 sec G-loads ranging from 1.5 to 3.5 ± 0.5 units were set up, and during reproduction of weightlessness, the G-load along the y axis did not exceed 0.02-0.07 units, and along the x axis, 0.002 unit.

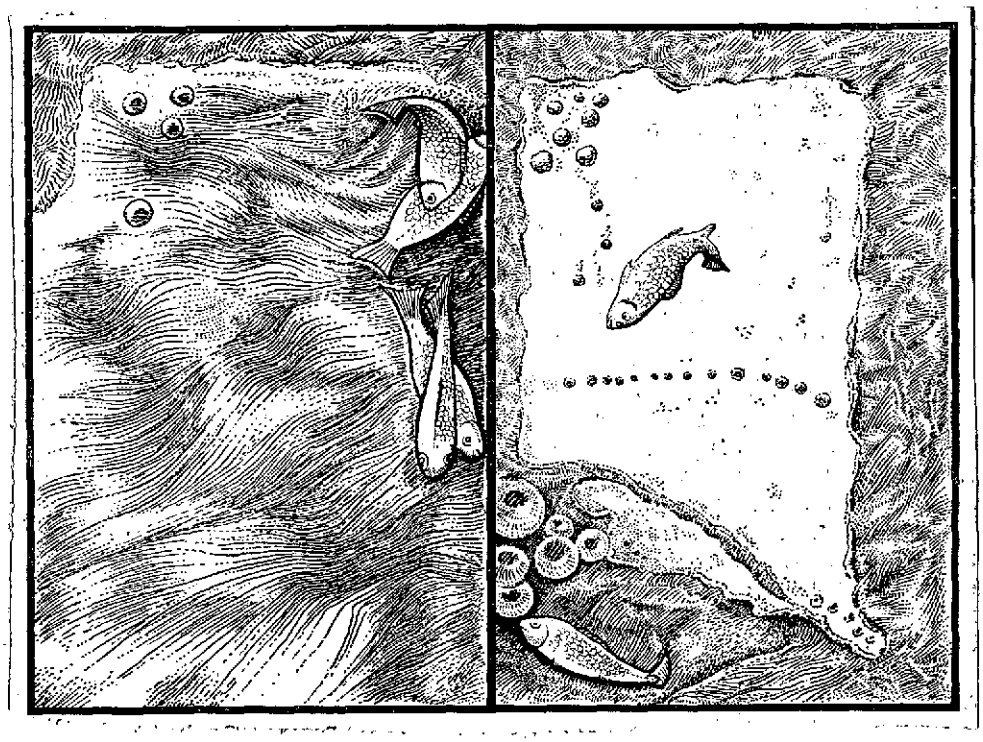
At the moment of transition from the action of the G-load to weightlessness, a considerable change was observed in the behavior of the fish. In compartment A as well as in compartment B, the fish lost the position of the body that is proper under conditions of normal weight. They swam in any position (on the side, head down, belly up, etc.), the speed of their swimming motions in compartment A not increasing appreciably, while in compartment B, it increased considerably. Moreover, in compartment B, owing to a still greater reduction in the water level (the water in weightlessness distributed itself along the walls of the aquarium), the fish emerged into the air in virtue of acute contraction of the musculature of the body and collision against the bottom or walls of the aquarium. In the air, 800 times less dense than water, the fish completely lost their customary swimming motions. The flight of the fish in the air was characterized by a number of chaotic "flapping" motions with complete loss of the body's orientation.

Emerging from the water and turning somersaults in the air, the fish, after reaching the upper part of the wall or the roof of the aquarium and bouncing back off it, returned to the water, where their motions slowed down a little but did not change in character (see figure). Under conditions of weightlessness, the behavior of the fish in our experiments was similar to that of small laboratory animals (white mice, rats), as described by V. I. Yazdovskiy, YE. M. Yuganov and I. I. Kas'yan [11], B. G. Burgov et al. [2], and L. G. Kitayev-Smyk [6].

As V. I. Yazdovskiy et al [11] write: "from the first moment and up to the end of the action of weightlessness, the white mice executed disordered 'somersaults' along the longitudinal, saggital and frontal axes" (p. 765). The same picture was observed in the case of the fish in our experiments, but, in contrast to the mice, which executed rotary motions with a practically constant rate of rotation (1.5-2 rps), the fish displayed no such motions.

In experiments with fish in weightlessness (lasting up to 8 sec), carried out on blinded and intact fish by Baumgarten et /24

al. [12], it was found that the fish reacted very quickly (in 0.5-1.5 sec) to a decrease in the gravity-force load by tilting their trunks downward and plunging to the bottom of the aquarium. The same authors established that the plunging of the fish incident to a reduction in the gravity-force load is not a compensatory reaction to the concomitant dilation of their swimming bladder. While the mice after 40-45 sec displayed adaptive reactions involving adjustment of body and motion under conditions of weightlessness, in our experiments, which lasted 20-25 sec, no adaptation to conditions of weightlessness could be observed. It is probable that in the case of fish, too, adaptive changes ought to be expected for longer-lasting weightlessness.



Compartment A Compartment B

Behavior of fish under conditions of short-lived weightlessness (drawing from motion picture frame). In compartment B, which was 1/10 filled with water, the fish emerged into the air.

Frame-by-frame analysis of the film we took during the experiments (random sampling of three out of the 68 experiments carried out) enabled us to work out the trajectory and calculate the rate of movement of the fish in both compartments of the aquarium in weightlessness.

In compartment A, which was almost completely filled with water, the maximum rate of movement of the fish in water did not exceed 20 cm/sec. The rate of movement in the first 5 sec dropped sharply to zero and, after 2-3 sec, fluctuated periodically from 0 to 15 cm/sec every 2-3 sec throughout the entire experiment. In compartment B, 4/5-1/10 filled with water, the rate of movement of the fish increased sharply, reaching in the 5th-6th sec of the experiment 30-40 cm/sec, and then also dropped periodically to zero every 2-3 sec. The maximum rate of movement of the fish in air in weightlessness came to 70 cm/sec.

The reduction in the rate of movement of the fish, and, consequently, their motor activity as well, in the first 5 sec of weightlessness is contrary to that which was observed in mice, rats and dogs [5, 11]: the motor activity of the animals in this period of weightlessness increased sharply. This behavior can be explained by a specific reaction of the fish organism, its nervous system. In fish in weightlessness, as in all animals, not only are the functions of the otolithic apparatus reduced or shut off, but the functions of the baroreceptors of the lateral line are also sharply reduced inasmuch as in weightlessness the hydrostatic pressure of water drops to zero. Thus, fish lose their orientation from their depth of immersion. All of this taken together sharply reduces sensory afference and destroys the functional interaction of the afferent systems, which leads in the first seconds of weightlessness to a sharp slowing down of motor reactions. /25

Subsequently, motor reactions are increased, as in all animals, as a result of the manifest incompatibility of reflex contractions of the muscles, which is adequate for normal or increased gravitation, with the state of weightlessness [11].

In our opinion, these experiments with fish in weightlessness constitute still another confirmation of the thought of V. I. Yazdovskiy, Ye. M. Yuganov and I. I. Kas'yan [11] that "the dissimilar degree of motor activity in the initial period of weightlessness and the different adaptive resources of animals may point to the individual [One might add, different in evolutionary origin -- V.M.] sensitivity of their neuroregulatory apparatus to the absence of the force of gravity, the different functional mobility of their nervous structures" (p. 766).

In our experiments with fish lasting 20-25 sec we were unable to detect any adaptive manifestations to weightlessness. Nor is this surprising inasmuch as for determining an organism's adaptation time, as P. K. Isakov, Ye. M. Yuganov and I. I. Kas'yan think, "incident to the action on it of acceleration and weightlessness, it is necessary to observe two conditions: the periods of action of these factors must be incomparably longer than the transitional period [short-lived weightlessness alterating with

g-loads -- V.M.], and the magnitude of the acceleration must not exceed that which is usual under terrestrial conditions by more than a factor of 2."

The disturbance of certain functions (especially of the cardiovascular system), which it is reasonable to assume to be possible in animals and man in prolonged weightlessness, will probably not be serious in the case of fish. And not because fish are less highly-organized animals, with a low sensitivity of their organism's systems, or because under normal terrestrial conditions they find themselves in water in a suspended state "as if in weightlessness." In fish there will be no serious shifts in the functioning of their organism's systems because in their aqueous medium they are evolutionarily adapted to low expenditures of energy in overcoming gravitational forces.

Of interest in this connection is the idea put forward by P. A. Korzhuyev [7] to compare the adaptations of terrestrial and aquatic animals for the purpose of overcoming gravitational forces. As compared with aquatic animals, the power of the foci of hemoglobin synthesis in terrestrial animals increased ten-fold and one hundred-fold in connection with their increased expenditures of energy, for the release of which considerable amounts of oxygen are necessary.

Thus, it is to be expected that aquatic organisms (including aquatic mammals) will be able to endure prolonged weightlessness with considerably greater ease than terrestrial animals and man.

Abstract

Under conditions of short-lived (up to 25 sec) weightlessness, fish lose their orientation in space and swim in any position of the body (on the side, head up, belly down, etc.). In aquaria that were 1/5-1/10 filled with water, in weightlessness fish were observed to emerge into the air, where they completely lost their customary swimming motions. The flight of fish in the air turns into chaotic "flapping" motions with complete loss of the body's orientation in space and a considerable increase (by a factor of 2 to 3) of the rate of movements. In the course of 20-25 sec of weightlessness, no adaptation to it was observed. Twelve references and one figure.

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MULTICHANNEL ELECTROSTIMULATION. STRUCTURE OF THE MULTICHANNEL SIGNAL AND OF THE APPARATUS

E. K. Kazimirov, L. Ya. Danilenko, and A. G. Kanarovskiy

The history of electrostimulation dates back 2000 years, to /26
the time when electric fish began to be used in the treatment of a number of illnesses. Over the last 200 years or so of the development of electrical engineering and, more recently, radio electronics, many apparatuses for electrostimulation have appeared. But their functional structure has practically changed no more than their field of application: treatment of illnesses and study of electrophysiological processes in living organisms.

At the present time, a new sphere of application of electrostimulation is being contemplated: its effect on healthy people. It is natural for new ideas to be reflected in the development of apparatus. In the last few years, multichannel electrostimulation methods have taken root. At once there arose a question: how to organize the structure of a multichannel signal -- the amplitude-time relations of signals on different channels -- and more urgent became the problem of optimizing the choice of the signal's parameters and the characteristics of the output stages of the stimulators on each channel.

Multichannel electrostimulation has been mainly directed toward man's motor apparatus, toward the different groups of muscles that differ in the nature of their participation in motion as well as in their parameters of excitability. The structure of the multichannel signal ought to reflect these peculiarities, and its action ought to measure up to any degree of complexity of the stereotype of operation of the motor apparatus.

An apparatus has been worked out for research purposes as well as for practical utilization. As a starter, let us dwell on the first form of the apparatus. The raw materials for building up the structure of the multichannel signal incident to its action on the motor apparatus is electromyograms of the fundamental muscles participating in motion -- the so-called EMG portrait of motion which, as has been shown in the studies of many authors, only approximately reflects the true picture of the coordinated interaction of the muscles. Therefore, the main requirements of a multichannel electrostimulator for research purposes are the possibility of forming an arbitrary signal structure and the simplicity of reorganizing this structure in the course of action.

Existing methods of forming a signal structure do not meet these requirements [1, 4, 8]. In the widely-known method of

multichannel electrostimulation controlled by signals from the electrical activity of the "donor's" muscles, the signal structure is strictly attached to the EMG portrait of the control signal. Moreover, because of signal distortions caused by the finite sensitivity of the recording apparatus and by transformations in the channel of formation of the electromyogram's envelope, the presence of an excitability threshold of the muscle and individual differences in "donor" and "donee", the structure of the actuating signal differs substantially from the original EMG portrait. /27

We worked out a method of organizing the structure of the multichannel signal (Figs. 1 and 2) that meets the above-mentioned requirements, and a device has been built that implements this method.

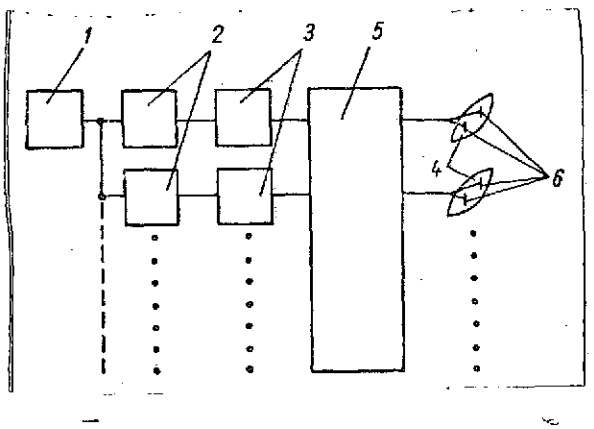

Fig. 1. Diagram showing multichannel electrostimulation method.

The electric pulse generator 1 (Fig. 1) generates the stimulation-triggering signal, which is common to all control channels, following the interval that is necessary for the given signal structure (cf. Fig. 2, a). In the concrete device that implements the given method, the interval is smoothly controlled within the limits from 0.2 to 10 sec. This pulse enters the delay assemblies 2, which are separate for each channel; here the controllable delay of the stimulation-triggering signal is brought about (cf. Fig. 2, b). The delay assemblies give the signal's phase structure. In the real device, the delays are smoothly controlled within the limits from 0 to 10 sec.

Further, started up by the electric pulses that are separately delayed in each control channel are the electric function generators 3, with the aid of which are generated in each control channel the stimulating signal's envelopes, whose shapes can be controlled (cf. Fig. 2, c). These envelopes determine the width of the action zone and the law of change of one or more parameters of the succession of stimulating pulses within the zone.

Separately formed for each neuromuscular unit 4, the envelopes are presented to the corresponding input of the multichannel stimulation block 5 with outputs that are uncoupled by galvanization. At the output of the stimulator we obtain the signal shown in Fig. 2, d. In the given case, the envelope determines the law of ch

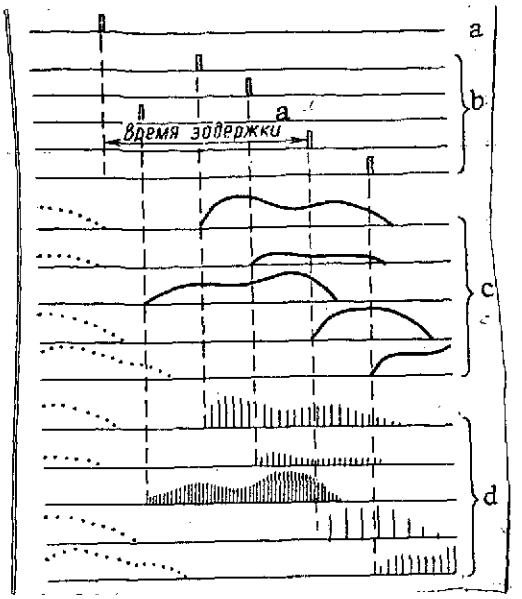

Fig. 2. Formation of the multichannel stimulating signal's structure.

Key: a. Delay time

of change in the pulse amplitude. In the real device, there is the possibility of obtaining separately the frequency modulation of the pulses according to the law of envelopes and joint amplitude-frequency modulation. The pulse parameters in each channel (shape, width, repetition period) are established independently of one another. The multichannel stimulating signal formed in this manner with the aid of the surface electrodes 6 are presented to the neuromuscular units 4.

This enabled us to accomplish our main task -- specification of an arbitrary structure of the multichannel stimulation signal and its reorganization during stimulation -- and begin research on the effect of the parameters of the signal structure on the electro- /28
stimulation effect, in particular, control of movements with high accuracy. As a result of the investigations, we must get closer to a conception of optimal signal structure that is applicable to one or another problem of action on selected complexes of the excitable structures of living subjects.

Into the described multichannel electrostimulator, two new parameters are introduced: the signal shape and the controllable output resistance. Selected as signal shapes were shapes that have been "evaluated" in the literature as influencing the character of the action, for example, pulses with a radial frequency priming, conjugate heteropolar pulses, etc.

The output resistance of the electrostimulator is an important factor determining the character of the interaction between the electrostimulator and the excitable structure. The attempt to apply electrostimulators in biological and medical research gives no unambiguous answer to the question of what is the most expedient type of output stage for the electrostimulator; in other words, the role of the output resistance [3].

Known are attempts to build electrostimulators with an output resistance that varies smoothly within wide limits [7]. In this case, however, the output resistance is conserved neither in the pauses between stimulating pulses nor during the transmission of their fronts -- which is a considerable shortcoming in the case of electrostimulation [illegible]

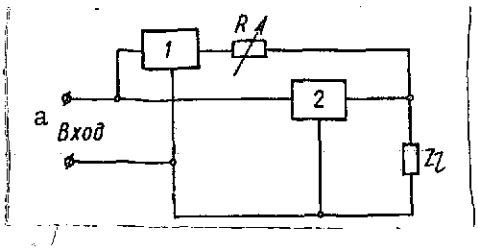

Fig. 3. Design principle of output stage with controllable output resistance.

Key: a. Input

pulse electrostimulators: the important influence of precisely the initial part of the pulse on the action effect is known.

We investigated in detail two variants of the output stage with a controllable output resistance. The first of them involves utilization in the output stage of a new circuit element of the rotator, with the aid of which it is possible to bring about a reversal relative to the coordinate axes of the volt-ampere characteristics of the transistor [5, 6]. By changing the angle of rotation within the limits of 0 to 90°, we can obtain a change in the output resistance from 400 kΩ to 40 Ω.

The circuit of the output stage on the rotator turned out to be rather complicated. The second variant, which makes use of the principle of summation for one and the same load of the effects from two independent amplifiers, proved to be simpler and more reliable. One of the amplifiers has a high output resistance (current generator) and the other, a low one (voltage generator). The operating principle of the output stage is clear from Fig. 3. Here 1 is the amplifier with the low output resistance, R_{i1}, and 2 is the amplifier with the high output resistance R_{i2}; i.e. satisfied is the condition

$$R_{i1} \ll Z_l \ll R_{i2},$$

where Z_l is the total load.

Amplifier 2 is connected with the load immediately and amplifier 1 through the controllable resistance R. The input signal enters the inputs of both amplifiers. For the output resistance we can write

$$R_{output} = R_{i2} \| (R + R_{i1}) = \frac{R_{i2}(R_{i1} + R)}{R_{i2} + R_{i1} + R}.$$

inasmuch as $R_{i2} >> R_{i2}$, when $R \to \infty$, $R_{output} \to R_{i2}$ and when $R \to 0$, $R_{output} \to R_{i1}$. The range of variation of the output resistance is practically given by the value of R_{i1} and R_{i2}.

For the voltage on the load U_{output}, we can write:

$$U_{output} = U_1 + U_2 = \frac{U_0 Z_l}{Z_l + R} + \frac{I_0 Z_l R}{Z_l + R} = f(R),$$

where U_0 is the output voltage of amplifier 1; I_0 is the output current of amplifier 2; U_1 and U_2 are the voltages for the total load from amplifiers 1 and 2, respectively. /29

When the condition $I_0 Z_l = U_0$ is satisfied, the output voltage does not depend on the value of the resistance R throughout the range of its variation and is equal to U_0. Indeed, substituting $I_0 Z_l = U_0$ in the preceding expression, we get

$$U_{output} = \frac{U_0 Z_l}{Z_l + R} + \frac{U_0 R}{Z_l + R} = U_0 \frac{Z_l + R}{Z_l + R} = U_0.$$

A concrete case of the output stage is described in work [3].

For practical utilization we developed electrostimulators in a portable execution. The simplified signal structure in the portable electrostimulators to a certain extent reflects the vagueness of our ideas about the role of the signal structure incident to multichannel action in general. The structural diagram of the portable electrostimulator is shown in Fig. 4. The electrostimulator contains a generator 1 of modulating oscillations, whose period is determined by the controller 2 of the oscillation period. The generator 1 generates signals of a sinusoidal shape. The signal from the generator 1 acts on the phase shifter 3, which is intended for varying the phase of the modulating signal in each channel. The phase shift angle of the modulating oscillation is determined by the controller 4 of the phase-shift angle in each channel.

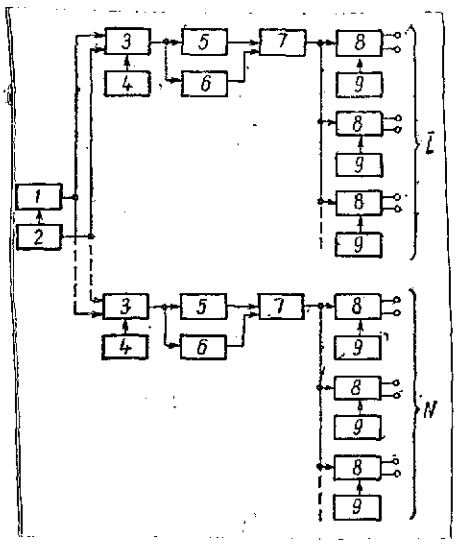

Fig. 4. Block diagram of the portable multichannel electrostimulator.

To conserve the magnitude of the phase shift angle incident to a change in the oscillation period of the generator 1, the controller 2 of the oscillation period is connected with the phase shifter 3 for the purpose of correction. With the aid of the frequency modulator 5 and the amplitude modulator 6 in each channel is brought about the cophasal frequency and amplitude modulation of the signal from the generator 7 of the signal-controlling pulses with the phase shifter 3. The generator 7 generates pulses of a rectangular shape. The signal from it acts

on the former 8 of the stimulating pulses in every output group of each channel. To one controlling pulse corresponds one stimulating pulse, in which case the amplitude of the stimulating pulse is directly proportional to the amplitude of the controlling pulse. Thus, the frequency and amplitude of the stimulating pulses also vary according to a sinusoidal law.

The parameters of the stimulating pulses in every output group of each channel is determined by the controller and regulator 9 of the parameters of the stimulating pulses. The width and shape of the stimulating pulses and the amplitude of the stimulating signal are set and controlled. Within the limits of each channel it is possible to achieve several output groups that include the former 8 of the stimulating pulses with the controller and regulator 9 of the parameters of the stimulating pulses.

In setting the oscillation period we also set the total duration of the cycles of contraction and relaxation of the stimulated muscles. One part of the period of the modulating oscillations, for example, the positive half-wave of the sinusoidal ocillations, determines the cycle of contraction of the muscles and the other part of the period, the cycle of relaxation.

Entering the phase shifter 3 of all channels, the modulating signal of the generator 1 is shifted by an angle that is separately set in each channel of the controller 4 of the phase shift angle. Thus, the cycles of contraction and relaxation of the muscles are distributed in time over all channels and the required motor coordination is given. In the simplest case of stimulation of two antagonistic muscles, two channels are utilized, and the phase shift angle for each channel is given in such a manner that their difference will be equal to 180°. Then the cycle of contraction of one muscle will correspond to the cycle of relaxation of the other. /30

The signal from the generator 7 of the controlling pulses that is frequency and amplitude modulated by the signal from the phase shifter 3, enters the inputs of the former of the stimulating pulses in every output group of each channel. At the output of the former 8, we obtain the sequence of stimulating pulses with cophasal amplitude-frequency modulation according to a sinusoidal law; the parameters of these pulses are set in the controller and regulator 9 of the parameters of the stimulating pulses. The signal from the output of the former 8 of every output group of each channel enters the electrodes. The contraction of the muscle is caused by the pulses whose amplitudes are above the threshold of excitability; pulses with a greater amplitude and frequency cause a stronger contraction.

Introducing within the limits of each channel several output groups with separate assignment of the parameters of the stimulating pulses permits several muscles to be stimulated

cophasally with due regard for their individual characteristics, for example, the threshold of excitability and forces of contraction; i.e., if incident to a certain motor reaction there are muscles that operate cophasally but differ in their characteristics, they are switched into different output groups of one channel.

To form the stimulating pulses, the phenomenon of self-induction was utilized: the energy necessary for the pulse is accumulated after a relatively long period of time and is then discharged on the load for a short period of time, forming the stimulating pulse of the necessary amplitude and duration. This principle of forming the stimulating pulses permitted the galvanic uncoupling of the outputs to be brought about easily, the dimensions of the apparatus to be considerably reduced, the power consumption to be reduced, and a power pack of practically any voltage value to be used (for example, low-voltage accumulator batteries).

We developed and manufactured experimental models of an electrostimulator on two channels with five output groups in each channel. Below are presented the fundamental parameters of the device:

Type of output	Current generator
Pulse duration	1.0-1.5 msec
Current amplitude at each output	0-70 mA
Period of modulating signal	1-5 sec
Full frequency deviation of sequence of stimulating pulses	20-100 Hz
Percentage of amplitude modulation	$\geq$ 80%
Control of phase shift between channels	20-180°
Supply voltage from battery	9 V
Dimensions	250 x 150 x 65 mm
Weight with supply block from network	3 kg

Abstract

The authors describe the organization of an arbitrary reorganizable signal structure of multichannel operation, the output stage of electrostimulators with a controllable output resistance and a portable multichannel electrostimulator. Eight references and four figures.

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EFFECT OF 10- AND 30-DAY HYPOKINESIA AND ELECTROSTIMULATION ON SOME ACTIVITY INDICES OF THE CARDIOVASCULAR SYSTEM

I. V. Muravov, Ye. A. Pirogova, and O. M. Potetyunko

By increasing the power available per worker, scientific-technological progress is leading to a sharp reduction in man's motor activity -- hypokinesia. Of interest at the present time are mainly three fundamental aspects of this, one of the most urgent problems of the century: /31

1. Hypokinesia, in conjunction with sharply increasing stress on the psyche -- the analyzing system -- and with increasing intellectual activity, is setting limits to the functional resources of the human organism in its complicated interaction with the machine and is thereby becoming the limiting factor of present-day production.

2. Hypokinesia is characteristic of a great number of illnesses and, in view of the nature of the pathologic process (different diseases of the cardiovascular system, nervous breakdowns, traumas, etc.), it is inevitable that this condition should occasionally last a very long time.

3. Hypokinesia accompanies man's prolonged stay under space flight conditions, which entail considerable changes in the neuromuscular apparatus and a crucial reorganization of the system of reflexes that ensure the various vital functions.

Numerous experimental and clinical studies of the effect of hypokinesia on the functions of the organism have shown that the immediate result of evolving insufficiency of muscular activity is profound disturbances throughout the organism that lead to a generalized pathologic process [2]. In the complex of changes arising in consequence of a deficit of movements in the motor apparatus and internal organs, disorders of the cardiovascular system occupy the leading place [3, 11, 24, 28, 30, 32, 33, 34]. Under the influence of hypokinesia, the efficiency of the cardiovascular system at rest is reduced and the strain on it is increased, and the ability of the circulatory apparatus to adapt itself to the changing conditions of the external environment also deteriorates critically. The exceptional sensitivity of the circulatory system to the effect of unfavorable factors inspired V. V. Parin et al. [16] to regard it as a universal indicator of different disorders.

As a rule, what is examined in most studies devoted to the effect of hypokinesia on the functioning of the cardiovascular

system [3, 4, 6, 7, 10, 15, 17, 20, 21, 25, 26, 31] is the changes arising in a state of rest. The number of studies giving exhaustive information about the distinctive features of the adaptation of the circulatory system to muscular activity is extremely meager [1], and the available data are very contradictory. Nor have the possibilities afforded by prophylactic agents that might reduce or remove the effect of hypokinesia been sufficiently studied, even though this aspect of the investigations is acquiring an exclusively practical significance [9, 12-14, 27]. /32

The present study gives an account of the results of studying the distinctive features of the adaptation of the cardiovascular system to muscular activity under the influence of 10- and 30-day hypokinesia, as well as the effects of electrostimulation of the muscles of the extremities and trunk on the functional state of the circulatory organs under these conditions.

The studies were conducted on six male volunteers aged from 24 to 33 years, three of whom experienced the effects of 10-day and three of 30-day hypokinesia under conditions of strict bed rest. Four men (two per group) were subjected to electrostimulation; the other two served as control. To exclude those few movements that are possible during sleep, high, thick, plaster slings were put on the lower extremities of the examinees who were under the conditions of 10-day hypokinesia. The examinees made their toilet, took food and performed the natural functions while lying in bed. They were sometimes permitted to move their upper extremities and sometimes, to turn on their side, read, watch TV.

We passed judgment on the changes in the functional state of the cardiovascular system not by the end result of the reactions, but on the basis of their development and restoration incident to different types of physical loads and in the period of restitution. Inasmuch as it is known that changes in the functional state of the cardiovascular system are more pronounced at the moment of transition from rest to activity and vice-versa (from activity to rest), particular attention was paid to analysis of the "working in" period and to the changes in the next phases of respiration. For this purpose we evaluated the degree of shortening of a complex of five intervals R - R (we took into account the first, beside the costing and last complexes recorded during work, the first one that was fixed immediately after the physical load, as well as at the end of the first and second minutes of the period of restitution).

The muscles of the trunk of the upper and lower extremities were electrostimulated twice a day -- in the morning and evening -- with the aid of a PMS-1 device with a frequency of 60 muscular contractions per minute for a modulation period of 2-4 sec. In the course of the 10- and 30-day hypokinesia we carried out from two to six 25-30 min stimulation cycles consisting

of 4 days of stimulation and 1 day of rest. The force of the muscular contractions in the first 2 days amounted to 70-80% of the maximal force attainable on the third day, and on the fourth day it dropped to 40-50% of the maximal. In the morning we stimulated the muscles of the back and abdomen, the gastrocnemius muscles and the muscles of the posterior and anterior surface of the thigh; in the evening, the protibial muscles, the quadriceps femoris muscles, the deltoid muscles and the muscles of the neck.

For the test with physical loads, the examinees were asked to work on a wrist ergograph at the rate of 60 contractions per minute with a 13-kg load for 1 min and a 7-kg load until the examinee's absolute refusal to continue working, as well as to ascend a 25-cm high footboard for 3 min at the rate of 30 ascents per minute.

The results of the investigations show that under the influence of hypokinesia in a state of rest, in all examinees (those subjected to electrostimulation, as well as the control group), the cardiac rate increases sharply. It is interesting to note that in the examinees who were under conditions of strict bed rest for 10 days, the difference between the initial pulse rate before and after the influence of hypokinesia was somewhat less as compared with the examinees who were subjected to its influence for 30 days. This difference is particularly clear in the control group: 14 strokes for 10-day and 25 strokes for 30-day hypokinesia. In the examinees who were subjected to electrostimulation, these values were equal to 12 and 23 and 14 and 27 strokes, respectively. Our data agree with the results of the investigations of a number of authors [3, 5, 7, 18], who, in contrast to some other researchers [15] who detected a slowing down of the pulse under hypokinesia conditions, established a considerable increase in the pulse rate. /33

Shifts in the opposite direction were noted during the physical loads. Analysis of the changes in the pulse rate at 10-sec intervals in the process of muscular activity explains the reduction in the response -- a decrease in the increment in the cardiac rate to a 1-min standard load.

As can be judged from the data shown in the table, the nature of the adaptation of heart action to a muscular load in the examinees subjected to 10- and 30-day hypokinesia without electrostimulation and in conjunction with daily electrostimulation is in the main similar. Reactions of the same type, which indicates a decrease in magnitude of the shifts in the pulse rate during muscular activity, can also be observed incident to more protracted loads -- a 3-min ascent on the footboard and work until complete fatigue.

Moreover, in two examinees who had been subjected to 30-day hypokinesia and in one serving as control in the group that had

CHANGE IN THE INCREMENT IN THE CARDIAC RATE UNDER THE INFLUENCE OF 10- AND 30-DAY HYPOKINESIA UNDER CONDITIONS OF A STANDARD PHYSICAL LOAD (WORK ON AN ERGOGRAPH WITH A 13-kg LOAD)

Examinee	Nature of action	Research period	Initial frequency	Increment in frequency (in %) after 10 sec	20 sec	30 sec	40 sec	50 sec	60 sec
30-day hypokinesia									
P-s'	Rest	Before expmt.	74	17.5	20.2	17.6	21.5	21.5	22.8
		After expmt.	100	17.0	17.0	18.0	18.0	19.0	19.0
K-r	Electrostimulation	Before expmt.	86	19.7	20.9	22.0	20.9	23.2	23.2
		After expmt.	100	18.0	18.0	18.0	20.0	21.0	20.0
S-n	Same	Before expmt.	74	21.6	17.5	20.2	20.2	21.6	22.8
		After expmt.	101	17.8	16.8	18.8	17.8	17.8	19.8
10-day hypokinesia									
K-y	Rest	Before expmt.	61	22.9	24.7	27.8	29.4	29.4	31.1
		After expmt.	75	20.0	22.6	22.6	24.0	24.0	24.0
T-n	Electrostimulation	Before expmt.	58	18.9	18.9	18.9	22.4	20.7	22.4
		After expmt.	81	18.5	18.5	19.7	19.7	19.7	19.8
Kh-y	Same	Before expmt.	74	21.6	22.3	22.1	23.4	24.6	25.5
		After expmt.	86	20.9	20.9	20.9	22.8	23.2	22.8

experienced the action of 10-day hypokinesia, it was noted after prolonged performance with loads that the magnitude of the shifts was exceeded, although when performing with a standard load the reactions at first were substantially lower. This fact may point to an increased strain on the activity of the cardiovascular system, which is also confirmed by the increase in the period of restoration of the activity indices of the heart to its initial level, as well as by the appearance of "step-by-step" pulse reactions in all examinees after 30-day hypokinesia and in one examinee after 10-day hypokinesia.

The changes in the functioning of the cardiovascular system that develop under conditions of insufficient muscular activity become understandable when we take into account the results of investigations of animals [2, 14], which develop not only a worsened functional state of their cardiovascular system with a lengthening of the influence of hypokinesia, but also a shortening of their very lifetime. A lack of movements leads to a weakening of the trophic effects on the tissue and, as a result, to profound changes in the state of the neuromuscular apparatus and the activity of the internal organs, especially the heart, as well as to accelerated formation of the senile mechanism of regulation of the functional state of the tissues [23]. The changes in the activity indices of the heart at the start of muscular work indicate the development of its state of profound detraining. Our previous investigations [19] established that as a result of systematic execution /34

of physical exercises, the development of reactions is accelerated, the "working in" period is abridged and the time of restoration of the reactions to its initial level is shortened.

In the organism of healthy people, many days of hypokinesia give rise to shifts in the opposite direction, which are the more pronounced, the longer the duration of restricted mobility. Thus, while under the influence of 10-day hypokinesia, the restoration time of the pulse rate after ascending the footboard increased from 2-3 to 4 min, after 30-day hypokinesia this index increased from 2 to 13 min. Fig. 1 shows the substantial differences in the changes undergone by the indices that characterize the "working in" and restoration of reactions for hypokinesias of different durations.

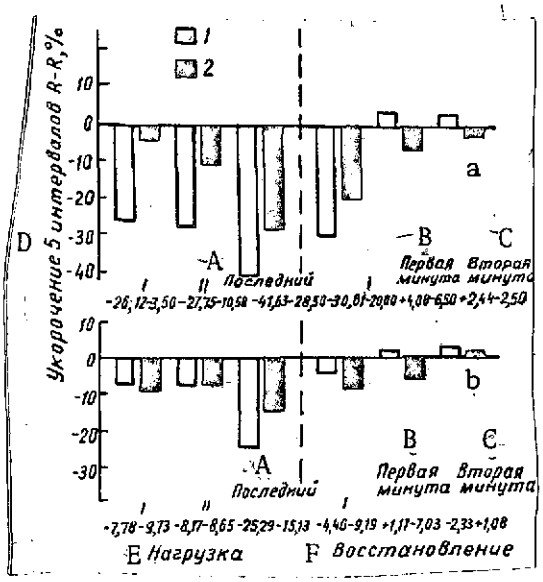

Fig. 1. Change in complex consisting of five intervals R - R under conditions of a physical load and during restoration: a. examinee K-r, 28 years old, 30-day hypokinesia; b. examinee T-n, 33 years old, 10-day hypokinesia; 1. before hypokinesia, 2. after hypokinesia; I. first complex; II. second complex, last - 5 intervals R - R at the end of work; 1. first complex during restoration; first minute - at the end of the first, second minute - at the end of the second minute.

Key: A. Last; B. First minute; C. Second minute; D. Shortening of intervals R - R, %; E. Load; F. Restoration time.

Analysis of the changes in the other important parameter of the functional state of the cardiovascular system -- the arterial pressure -- shows that at rest, many days of restricted mobility do not substantially change the magnitudes of the systolic and diastolic pressures. Some increase was noted in the systolic pressure (by 5-20 mm Hg) within the limits of the physiological shape. Under the influence of the load we adopted, these indices did not change in the same way in different examinees. What is more, as a result of 10-day hypokinesia, the magnitude of the shift in the systolic pressure incident to 1 min of work on the ergograph increased by 5-15 mm Hg, while for 30-day hypokinesia, on the contrary, it decreased by 10-25 mm Hg. As a rule, the diastolic pressure in both groups of examinees under these conditions did not change. But work to the point of complete fatigue caused a regular increase in the diastolic pressure by 5-15 mm Hg, and in

examinee P-s', who had not been subjected to electrostimulation, after 30-day hypokinesia the increment in this index increased by 20 mm Hg (before hypokinesia its reduction by 10 mm Hg was recorded).

Incident to ascent on the footboard, the shifts in the systolic pressure under the influence of hypokinesia in the majority of cases did not change. Only in two examinees did this index increase after the exercise: in one examinee, after 10-day hypokinesia, it rose by 40 as against 20 mm Hg before the experiment and in the other examinee, after 30-day hypokinesia, it rose by 50 as against 25 mm Hg. Moreover, in four examinees, the diastolic pressure rose by 5-20 mm Hg while in two others, on the contrary, it decreased by 15 mm Hg. Under these conditions, the invariance of the systolic pressure with increasing diastolic pressure caused an increase in the pulse pressure of most of the examinees.

Thus, the effect of hypokinesia when using different physical loads is reflected in ambiguous, and sometimes opposite, shifts in the systolic and diastolic pressures. A 1-min standard load /35 reveals the phase nature of the shifts in the systolic pressure, depending on the acting times of the hypokinesia. The distinctive features of the phase shift are an increase in this index under the influence of 10-day hypokinesia and contradictory reactions of the systolic pressure after 30 days of restricted mobility.

The nature of the shifts that develop in the circulatory apparatus during electrostimulation depends in the first place on the topography of the groups of muscles that the stimulation puts to work. When the muscles of the back, abdomen and lower extremities were stimulated, the shifts in the pulse rate were greater than when predominantly the muscles of the upper shoulder girdle and neck were stimulated (Fig. 2). With an increasing number of stimulation periods, the increment in the pulse rate increased at the time of stimulation. Thus, while the maximum increment in the pulse rate amounted to 2 strokes/min in examinee S-n, 28 years old, when the first electrostimulation session was held, after 2 weeks it rose to 14 strokes/min.

During electrostimulation, the shifts in the arterial pressure changed in the opposite fashion: at the start of the course, the maximum increment in the systolic and diastolic pressures proved to be higher than at subsequent stages of this procedure. In the same examinee S-n, for example, on the first day the maximum increment in the systolic and diastolic pressures amounted to 20 and 12.5 mm Hg, respectively; after 2 weeks, it amounted to 11 and 8, and at the end of the course, to 9 and 5 mm Hg.

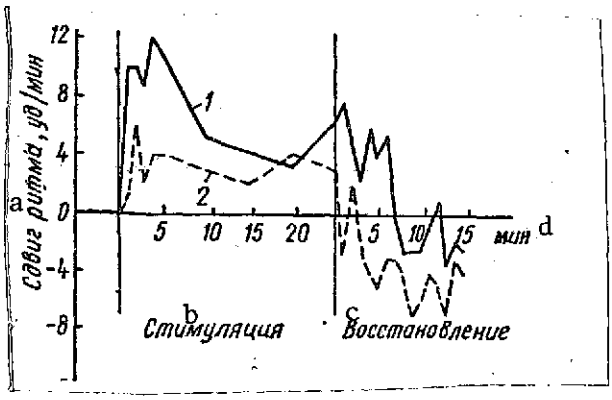

Fig. 2. Increment in the pulse rate and its restoration time versus the nature of the stimulated groups of muscles (examinee K-r, 28 years old). Stimulated muscles: 1. of the back, abdomen and lower extremities; 2. of the upper shoulder girdle, neck and protibia.

Key: a. Shift in rhythm, strokes/min; b. Stimulation; c. Restoration; d. min

Thus, by a number of signs, the effect of electrostimulation, for all its difference from the effect of physical loads, is similar to that of physical stresses. The absence of clear differences in the changes in the investigated indices of the cardiovascular system in examinees subjected to the effect of hypokinesia alone, as well as to hypokinesia and electrostimulation, does not entitle us to draw definite conclusions about the effectiveness of this method in the prophylaxis of hypokinesia. The fact that the signs of deterioration of the functional state of the circulatory apparatus appeared more frequently in examinees who had not been subjected to electrostimulation holds out promise for the application of this method as a means of preventing the unfavorable effect of restricted motor activity.

Abstract

Analysis of the development and restoration of the reactions of the cardiovascular system under conditions of 10- and 30-day hypokinesia disclosed the dependence of the adaptation of the circulatory function on the duration of restricted mobility. The nature of the adaptation of heart action to physical loads in persons subjected only to the action of restricted mobility is in the main similar to that in persons subjected to hypokinesia in conjunction with daily electrostimulation and attests to the development of signs of profound detraining. The fact that the signs of deterioration of the functional state of the circulatory apparatus appear less frequently in persons subjected to electrostimulation holds out promise for the application of this method as a possible means of preventing the unfavorable effect of restricted motor activity. Thirty-four references, two figures, one table.

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EFFECT OF HYPOKINESIA AND MUSCULAR OVERLOADS ON ANIMAL HEART ACTION

V. A. Boyer and A. G. Rakochi

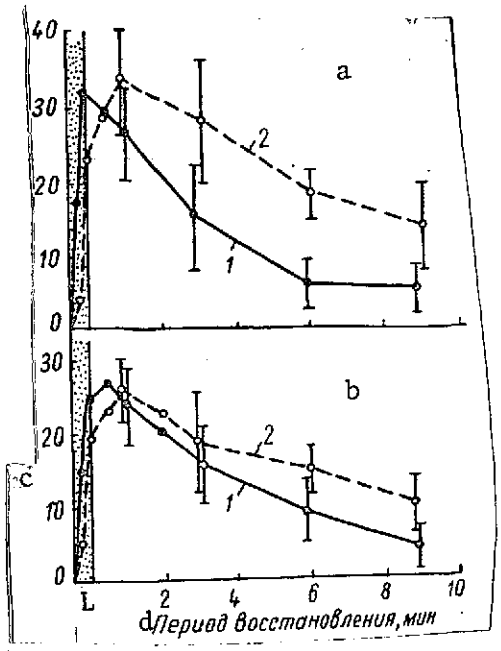
The problem of the effect of hypokinesia, isolation and physical loads on the functional state of the organism, so important in the research area of space biology and medicine, has been studied in different subjects -- in man and animals. Of considerable interest is the study of the functional changes in the animal organism under stress conditions, changes connected with different levels of motor activity. It has been established that the restriction of mobility, because of the drop in afferent input, acts in the initial stage like stress [3, 5, 6]. /36

But an increase in the motor activity of animals causes similar changes in the suprarenal gland, the thymus and the spleen [15]. /37

In view of the role of the cardiovascular system in ensuring the working capacity and viability of the organism, we have made an attempt to investigate the effect of hypokinesia and overloads on animal heart action. The experiments were conducted on 165 white rats aged from 6 to 8 months. The effect of hypokinesia was studied in 114 animals, and of muscular overloads, in 51 (including the control). Hypokinesia was created by placing the animals for 23.5 h a day in special cages. Muscular overloading was caused by running the rats daily in a treadmill until they gave up. The hypokinesia and overloading periods lasted 2 weeks. In view of the fact that the studies under different conditions were not carried out at the same time, to each test group of rats corresponded a separate control group made up of animals of the same age and sex kept under normal vivarium conditions. The control and test rats were in one room and received the same food ration.

Heart action was estimated from ECG data at rest and incident to a proportioned dynamic load (running in a treadmill) in unfixed animals adapted to the experimental conditions, as well as from the indices of the phase structure of the cardiac cycle under conditions of neuromuscular stress incident to fixation of the animal. The polycardiogram was recorded and interpreted according to Blyumberg's method as modified for small animals by Yu. A. Kuchak [8]. The weight of the animals' bodies and hearts were also determined (after sacrifice). The obtained data were processed by the methods of variance statistics.

As can be seen from the figure and Tables 1 and 2, under the effect of conditions of restricted mobility and muscular



Reactions of the rhythm of the heart under conditions of proportioned dynamic load and restoration: a. in "hypokinesthetized" animals; b. in "overtrained" animals; 1. control; 2. test; L. - load.

Key: c. Shift in rhythm of the heart, % of initial; d. Restoration period, min

overloading, unidirectional changes in heart action developed in the rats. Thus, in the animals of both groups, the cardiac rate at rest increases, the reaction of the rhythm of the heart and its restoration are slow to develop under a dynamic load and monotypic shifts in the phase structure of the systole take place incident to muscular stress. In the absence of reliable changes in the duration of the cardiac cycle and electromechanical systole, in "hypokinesthetized" and "overtrained" rats an increase in the absolute and relative duration of the stress period and a decrease in the expulsion period can be observed. Such a reorganization of the phase structure of the systole in the test rats during physical stress does not measure up to the load conditions and are evaluated as unfavorable inasmuch as a considerable part of the energy of the myocardium is uneconomically expended on preliminary work instead of on propulsive activity of the heart in expelling blood.

The results obtained (increase in the rhythm of the heart at rest, the nature of the curve of the pulse reaction incident to a load and unfavorable changes in the chronocardiogram) point to a decrease in efficiency and an increase in strain in the activity of /38 the cardiovascular system under the effect of restricted motor activity and under conditions of muscular overloading. The strain in heart action is indirectly corroborated by the increase in the heart's relative weight with decreasing body weight.

In the case of the test animals, to be noted is the increase in the phase duration of the asynchronous contraction, during which, as is known, the distribution of the excitation and the development of the contractile process take place in the myocardium. The phase duration of the asynchronous contraction depends on changes in the cardiac tonus that are connected with nerve activity, and is also determined by the characteristics of the metabolism of the myocardium, which affects the rate of distribution of the depolarization wave. Changes in the extracardiac heart control mechanisms

TABLE 1. CHANGE IN THE PHASE STRUCTURE OF THE SYSTOLE IN RATS UNDER THE EFFECT OF 2-WEEK HYPOKINESIA AND MUSCULAR OVERLOADS

Group	No. of animals	Phase duration, msec: Cardiac cycle M ± m	P	Electromechanical systole M ± m	P	Asynchronous contraction M ± m	P	Isometric contraction M ± m	P
Incident to hypokinesia									
1. Control	18	134 ± 4	> 0.05	70 ± 3	> 0.05	15 ± 1	< 0.05	13 ± 1	> 0.05
2. Test	11	141 ± 3		73 ± 2		18 ± 1		12 ± 1	
Incident to muscular overloads									
3. Control	12	146 ± 4	> 0.05	82 ± 3	> 0.05	19 ± 1	< 0.02	11 ± 4	> 0.05
4. Test	10	139 ± 4		84 ± 2		23 ± 1		11 ± 1	

	VSP, %: Stress period M ± m	P	Expulsion period M ± m	P	Stress period M ± m	P	Expulsion period M ± m	P	Mechanical coefficient $K_{M.A}$ M ± m	P	Systolic index, % M ± m	P
Incident to hypokinesia												
1	25±2	> 0.05	45±2	> 0.05	36,1±1,5	< 0.05	63,9±1,5	< 0.05	1,8±0,1	< 0.05	52,3±0,9	> 0.05
2	29±1		44±1		40,2±0,8		59,8±0,8		1,5±0,1		51,9±1,9	
Incident to muscular overloads												
3	29±2	< 0.1	53±2	> 0.05	35,9±1,9	< 0.05	64,1±1,8	< 0.05	1,9±0,1	< 0.05	56,5±1,0	< 0.1
4	34±2		50±1		40,7±1,3		59,3±1,4		1,5±0,1		60,0±1,4	

incident to hypokinesia [4] probably have a considerable effect on the course of metabolic processes in the cardiac muscle, which is in turn reflected in the development of electromechanical phenomena in the heart. Moreover, it seems that the decalcification of the organism that develops, according to the data of a number of authors [7, 9], incident to hypokinesia in man and animals may disturb the linkage of the excitation and contraction processes and in so doing, the intereonnection between electrical and mechanical phenomena in the myocardium. /39

The changes found in heart action indicate a disorder of the extracardiac control mechanisms. Thus, similar changes in the chronotropic function of the heart were detected by a number of authors under conditions when parasympathetic nerve influence on heart control were excluded or restricted. In chronically vagotomized animals have been observed: tachycardia at rest and a slow rise in the cardiac rate incident to a load [4, 13], an increase in the duration of the asynchronous phase, an increase in

TABLE 2. SOME ACTIVITY INDICES OF THE HEART OF RATS UNDER CONDITIONS OF HYPOKINESIA AND MUSCULAR OVERLOADING

Group	Rhythm at rest			Duration of restoration period			Relative heart weight*		
	No. of animals	M ± m, strokes /min	P	No. of animals	M ± m, strokes /min	P	No. of animals	M ± m, %	P
Incident to hypokinesia									
Control	48	358±4	<0.05	9	12,5±0,9	<0,02	26	0,34±0,01	<0.001
Experimental	37	378± 8		8	20,5±2,5		6	0,49±0,02	
Incident to muscular overloading									
Control	20	377± 7	<0,02	20	10,8±0,6	<0.001	17	0,29±0,01	<0,01
Experimental	9	420±14		6	16,0±1.1		9	0,35±0,01	

* In relation to body weight

absolute and relative durations of the stress period and a decrease in the expulsion period, and an increase in the systolic index. Slow restoration of the rhythm of the heart after a load has been shown in man under conditions of atropinization [10]. Observed in people under the influence of hypokinesia have been a slowing down of the rhythm of the heart during work and an attenuation of Aschner's reflex [4], and tachycardia at rest [16]. We may therefore assume that the changes in heart action that we noted under conditions of hypokinesia and muscular overloading are to a certain extent due to an attenuation of the cholinergic mechanisms of heart control.

An important role in the change in neural heart control incident to restriction of an animal's mobility is played by the reduction in the excitability of the upper vegetative centers that control the circulatory apparatus [8, 16]. According to our data [1], the attenuation of the parasympathetic influences on the heart incident to hypokinesia is connected with a change in the excitability not only of the central, but also of the peripheral parasympathetic structures that control heart action. In comparing the changes in the heart's excitability incident to reflex and direct stimulation of the vagus nerves under conditions of 2- and 4-week hypokinesia, we found a considerable restoration of the reflex bradycardiac reaction of the heart in mice after 4 weeks of hypokinesia as compared with the indices in animals subjected to 2 weeks of the same restricted mobility, although the thresholds of the electric effects incident to preganglionic stimulation of their vagus nerve remained increased. The threshold of excitability

incident to stimulation of the peripheral sections of the vagus nerves also rise under the influence of muscular loads: from 1.5 ± 0.1 V in the control to 1.8 ± 0.1 V in the experimental group ($P < 0.05$).

In mice subjected to 4 weeks of hypokinesia, the phase structure of the systole improved and body weight was partially restored [2]. Joint research with Z.P. Fedorova et al [12] showed that in these animals and in the controls, the number of erythrocytes and the magnitude of the hematocrit -- an indirect index of the mass of the circulating blood -- did not differ. /40

According to our data, the body weight of animals under conditions of protracted hypokinesia drops to a minimum in the first two weeks and is subsequently restored. The loss of body weight incident to hypokinesia is due not only to a disorder of metabolic processes, but also to dehydration of the organism, which in turn leads to a decrease in mass of the circulating blood, which, together with its redistribution in the organism, can alter the stimulation of the volumetric receptors of the vascular system that play, according to the data of Gauer and Henry [14], an important role in neurohumoral control of the circulation.

In view of the above-described concepts and the nature of the phase reorganization of the systole, we may assume that the worsening of the chronocardiographic indices that develops in mice incident to hypokinesia against a background of attenuation of parasympathetic neurocontrol is also connected with a decrease in mass of the circulating blood.

It has been established that stress situations similar to those studied in our experiment are accompanied by acute predominance of sympathicoadrenal influences on the heart, which seem to be due not only to an increase in the adrenergic background of heart control, but also to an increase in parasympathetic regulatory influences.

Restricted mobility and muscular overloads cause monotypic unfavorable changes in the activity of the cardiovascular system of animals. The similarity of the functional disorders incident to the action of these factors that are contradictory in a motor sense may be due, on the one hand, to the emotional stress that arises in the animals of both groups in connection with the experimental conditions and, on the other, to the relative drops in afferent input incident to restricted, as well as excess, motor activity.

Abstract

Study of the effect of hypokinesia and muscular overloads on the functional state of the heart of white rats revealed the

unidirectional nature of the changes in activity of the cardiovascular system, which indicates a reduction in the efficiency of heart action and a deterioration of the adaptation of the circulatory apparatus to loads.

The similarity of the functional changes incident to the action of factors that are contradictory in the motor sense is due to the monotypic reaction of the circulatory apparatus to stresses of different origins. Twenty-four references, one figure, two tables.

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EFFECT OF NOISE AND VIBRATIONS ON THE DEVELOPMENT OF EXPERIMENTAL NEUROGENIC GASTRIC ULCERS

N. P. Mashchenko and G. N. Lipkan

Not only workers from a number of industries are exposed to /41
the effects of noise, vibrations and their complex action, but also spacecraft crews. The study of the effect of these factors on the organism is therefore of indubitable interest for professional pathology as well as for space biology and medicine.

In the present study, the effect of noise and vibrations on the development of experimental gastric ulcers is studied. As experimental animals we chose white rats weighing 240-400 g kept on an ordinary vivarium diet. Of the great number of models of how to produce experimental gastric ulcers, we opted for the immobilization of the animals. According to the data of Bonfils et al. [4] and Brodie et al. [5], in rats the frequency of gastric ulcerations caused by 24-hour immobilization amounts on the average to 86-88%. As S. V. Anichkov and his collaborators [3] point out, the formation of dystrophic lesions of the stomach incident to immobilization of rats is such a constant phenomenon that it can be made use of in producing experimental ulcers and in their pharmacotherapy. This model attracted our attention because, according to the data of several authors [2], the gastric ulcers that arise in animals as the result of artificially induced neurosis are closest of all in their origin to the gastric ulcers of man incident to neurogenic forms of gastric disease.

Neurogenic ulcers were caused in the control by means of forced immobilization of the rats that had been starved for 2 days. The rats were tied to a plank by their legs for 24 hours. The experimental animals were subjected in a closed, soundproof room to the action of noise of a general intensity of 90 dB and to vibrations with a frequency of 50 Hz and an amplitude of 2-3 mm -- or the combined action of noise and vibrations of the indicated parameters for the first 3 hours of immobilization. After 24 hours, the control and experimental animals were sacrificed and opened. The stomach was extracted, cut along the great curvature and spread out on a glass plate. The changes that arose were studied macroscopically. The number of ulcers that formed on the mucosa of the stomach of each rat was computed and their size was estimated in points. To 1-2 mm ulcers was assigned 1 point and to 2-10 mm ulcers, 5 points. The ulcerogenic effect was estimated according to the Pauls index [6]

$$\text{Pauls index} = \frac{\text{Degree of ulceration} \times \% \text{ mice with ulcers}}{100}$$

Instead of the degree of ulceration, which usually indicates the average number of ulcers per rat, in our calculations, following A. P. Akimov [1], we used the area of ulceration of the mucosa of the stomach in points, which reflects more objectively the degree of dystrophic lesions. As can be seen from the data below, noise and vibrations have a pronounced effect on the development of neurogenic gastric ulcers in rats, intensifying formation of ulcers of the mucous membrane. /42

	Number of rats	% rats with ulcers	Average area of ulceration (in points per rat)	Pauls index
Control	7	86	7.4	6.4
Noise	6	100	18.8	18.8
Vibrations	6	100	36.7	36.7
Noise + vibrations	6	100	40.7	40.7

The Pauls index in the group of animals subjected to the action of noise increased as compared with the control group by a factor of 2.9; incident to the action of vibrations, by a factor of 4.2; and incident to the combined action of vibrations and noise, by a factor of 6.4. The groups are also distinguished by the nature of the dystrophic changes. In the control group, the ulcers were unitary, small and looked more like erosions of the mucous membrane. Autopsy revealed a white mucosa, without very pronounced swelling. Ulcers arose in only 86% of the animals. Incident to the action of noise, edema of the mucosa is more pronounced; a hemorrhagic content puts in an appearance; the ulcers are more distinct, yet do not exceed 2 mm (all were evaluated in points); their number increases. As a result of the action of vibrations, the large round ulcers with undermined edges that appear are elongated and situated along the course of the folds of the mucosa; they can be as large as 5 mm. The mucosa is reddish purple, edematous. The parallel action of noise and vibrations causes still greater dystrophic damage. In most of the experimental animals, all of the mucosa, which was covered with a viscous hemorrhagic content that could be washed off only with difficulty, is edematous, with many fine ulcerations and large round ulcers with undermined edges; there are many whitish points in the mucosa -- a sign of ischemia and dystrophy, which, however, take place without ulcerations. We did not take into account these changes, bordering on ulcers; but their presence and multitude compel our attention.

Thus, noise and vibrations considerably intensify the development of the experimental neurogenic ulcers that are caused by

immobilization. Most authors distinguish three basic factors in the mechanism of development of experimental gastric ulcers caused by immobilization: 1) the digestive action of the sour gastric juice; 2) vascular disorders; 3) a neural factor. Obviously, under the action of vibrations and noise, it is the last two that acquire special significance. In connection with the fact that the model we adopted is close to the human ulcerous disease of neurogenic origin, the data we obtained point to the possibility of easier development of an ulcerous disease in people subjected to the action of noise and vibrations.

Abstract

In experiments on white rats, the ulcerogenous action of noise and vibrations was studied against the background of immobilization of the animals, which was evaluated according to the Pauls index. It was established that noise and vibrations intensify the formation of gastric ulcers in rats. When the action of noise was associated with that of vibrations, more pronounced changes could be observed in the mucous membrane of the stomach. Six references.

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THEORETICAL FOUNDATIONS OF THE EFFECTIVENESS OF USING PHARMACOLOGIC SUBSTANCES AND OTHER MEASURES IN THE PROPHYLAXIS OF MOTION SICKNESS

R. I. Syabro, N. A. Razsolov, and V. A. Golovko

Studies by a number of foreign [20-26] and domestic [2, 3, 5, 11-19] scholars have established that labyrinthine and extra-labyrinthine influences participate in the pathogenesis of motion sickness, and motion sickness can appear in an overt or a covert form [6, 7]. /43

Starting from modern views of the reflex regulation of the vital functions [1], we may assume that in the formation of motion sickness reactions, afferent, central, effector and reverse afference links participate that close reflex arcs in the ring of functional systems at different levels of regulation. In conjunction with humoral factors of regulation, pulsation round the ring of functional systems ensures homeokinesis constants, the completeness of the organism's reactions and their self-regulation.

From this methodological point of view, motion sickness can be regarded as a kind of disturbance of the self-regulation of the organism's functional systems. Consequently, all measures to prevent motion sickness must be directed toward ensuring normal self-regulation of the homeokinesis constants of the functional systems in their different links: afferent, central, effector and reverse afference links.

What prophylaxis is chosen for motion sickness will depend on the category of persons subjected to accelerations. For persons executing, during or immediately after the action of accelerations, specific work (astronauts, aviators, pilots, radio operators, parachutists, etc.), in the foreground of measures for motion sickness prophylaxis must be training of the functional systems of the analyzer by different physical stresses, supplemented by suggestive, dietary and other measures. Autogenous training ought to occupy a special place in the prevention of motion sickness, but, unfortunately, it is seldom used for this purpose. The creation of a working dominant in the form of muscular stress and active cortical activity during the action of accelerations has proved itself by inhibiting illusions in the state of weightlessness. The pharmacologic effects must not disturb the physical or psychological working capacity of the given category of person. The selectivity with which pharmacologic substances act on separate links of self-regulation makes it possible to make use of preparations with different profiles of action.

The best-founded theory of the pathogenesis of motion sickness is the "otolithic" theory of V. I. Voyachek, which was further developed by his students and followers. Recently, Wood and Graybiel [25] of the USA Space Medicine Center, on the basis of testing discrete and complex preparations on people in a slowly rotating cabin and further checking them for the prevention of motion sickness on the sea and in the air, came up with a new theory of motion sickness. In the opinion of these authors, motion sickness sets in as a result of stress, which leads to a disturbance of the balance between the cholinergic and adrenergic structures of the CNS. They bolster their theory with data on the better effectiveness of a complex preparation containing substances with an anticholinergic (scopolamine, 0.6 -1.2 mg) and an adrenergic (phenamine, 10-20 mg) effect.

The conception of competing cholino- and adrenoreactive structures in the CNS can to a certain extent explain a number of symptoms of motion sickness and the effectiveness of the adrenomimetics and cholinolytics in the prevention of motion sickness, but it does not exhaust its possible mechanisms. From the standpoint of the whole organism as a complex, integrative, functional system, they are more complicated. That is why no single complex /45
preparation is effective in 100% of the cases. Motion sickness occurs in "vagotonic" as well as "sympathetic" types, and the effectiveness of one and the same preparation is not the same in different people.

Experimental data indicate that not only adreno- or cholinoreactive neurons participate in the reception, processing and transmission of sensory input to the effector organs, but also neurons of another mediation in different structures of the CNS that simultaneously play an important role in regulating the functions that ensure the life of the organism. It is inadmissible to count on the complete inhibition or occlusion of the central neurons that participate in the reception, processing and transmission of sensory input.

Nor must we neglect the effect on an effector link -- the smooth musculature, which is also a source of reverse afference. But even under these conditions none of the proposed complex preparations is effective in 100% of the cases.

The effectiveness of motion sickness prophylaxis with different substances and complex preparations varies, depending on the nature of the motion sickness, the sensitivity of the selected persons and the methods of testing and evaluating effectiveness. According to the data of American authors [20, 24, 26], the effectiveness of different preparations against motion sickness varies from 0 to 80%, depending on the preparation, testing conditions, approaches to their evaluation and, unconditionally, the sensitivity of the examinees. The complex preparations

COMPARATIVE EFFECTIVENESS OF MEDICINAL PREPARATIONS IN THE PROPHYLAXIS OF EXPERIMENTAL MOTION SICKNESS*

Preparations	Dose, mg	Effectiveness, % according to: our data	[8]	[24]
Discrete preparation				
Scopolamine (hyoscine)	1,2	100	100	100
	0,6	—	—	112
Aminazine (chlorpromazine)	25	—	—	17
	50	73	—	—
Dimedrol	50	—	58	—
Trilafon (etaperizine)	8	—	65	—
Eleuterokokk	30	—	81	—
Cyclizine	50	—	95	44
Stemetil	10	—	103	—
Haloperidol	3	—	107	—
Phenamine (amphetamine)	10	—	73	—
	20	117	—	—
"Vzletnaya" caramel	—	171	—	—
Dibazol	20	175	—	—
Pipolphene	25	203	—	—
Medicinal mixtures				
Caffeine b.n.	200	86	—	—
Barbitol n.	1000			
Scopolamine	1	—	96	—
Stemetil	10			
Plavephine	1 tablet	143	—	—
Scopolamine	1			
Cyclizine	50	—	147	—
Phenamine	10			
Scopolamine	1			
Cyclizine	50	—	150	—
Phenamine	5			
Scopolamine	1	—	154	—
Cyclizine	50			
Scopolamine	1			
Cyclizine	50			
Phenamine	5	—	155	—
Prozerine	10			
Pheplavine	1 tablet	186	—	—
Pipolphene	25	262	—	—
Caffeine b.n.	150			
Pipolphene	25	377	—	—
Phenamine	10			
Scopolamine	0,3	—	—	173
Phenamine	5			
Scopolamine	0,6	—	—	183
Phenamine	10			
Scopolamine	1,2	450	—	255
Phenamine	20			

* Placebo effect excluded.

"plavephine" and "pheplavine" that we have proposed had a protective effect in experimental motion sickness in swinging people subject to motion sickness in 73.9 and 62.5% of the cases, respectively, and with the addition of Coriolis accelerations, in 57.5%. Under natural conditions of motion sickness on the sea and in the air, their effect increased to 85-96% (excluding the placebo effect). Luminal-caffeine [2] and diphasine-dimedrol-caffeine [2] mixtures under maritime conditions had an effect in 81-85% of the cases. Unfortunately, the principle of evaluating the effectiveness of the preparations that is used by some authors [8-10, 25] renders impossible objective comparison of the data they obtained.

In comparing the effectiveness of individual medicinal substances and their mixtures in the propylaxis of experimental motion sickness (induced by different methods), taking the effectiveness of scopolamine in every investigation to be 100% (see table), it can be seen that even with such an approach, the effectiveness of identical preparations and their mixtures is not the same. The difference in effectiveness is connected with different experimental conditions, different sensitivities of the contingent of examinees to motion sickness, and different approaches to evaluating effectiveness. To obtain reliable data it is necessary to carry out comparative tests of all of the most effective complex preparations by the method of double blind control, using a placebo under identical test conditions according to a single method of evaluating effectiveness in one and the same group of people. The data obtained must be made the basis of recommendations for the use of the preparations in practice.

Abstract

Motion sickness is regarded as a form of disturbance of the self-regulation of the functional systems under the influence of labyrinthine and extralabyrinthine stimulation. Motion sickness prophylaxis must be conducted with the aid of complex pharmacologic preparations that act on central, effector and reverse afference links in conjunction with formation of a dominant, extero-interoceptive stimulations, autogenous training and hygienic measures.

A comparative analysis is made of the effectiveness of the preparations and their compounds in the prophylaxis of experimental motion sickness according to the latest data of foreign and domestic researchers. Twenty-six references and one table.

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DISINFECTION AND PRESERVATION OF REGENERATED DRINKING WATER BY SILVER

L. A. Kul'skiy, V. A. Slipchenko and O. S. Savluk

To provide astronauts with high-quality water for drinking and sanitary purposes is one of the most crucial challenges to crop up in solving the problem of safeguarding the life of man in space flights. As the period of autonomous existence of spacecraft is lengthened and the number of crew members is increased there develops a need for large stores of water, whose weight considerably exceeds the weight of air and food stores, taken together. Proposed in this connection have been different methods of regenerating drinking water from the condensate of atmospheric moisture and from the fluid of electrochemical generators, urine and other highly concentrated solutions, as well as from moisture-containing waste products [7, 9]. /46

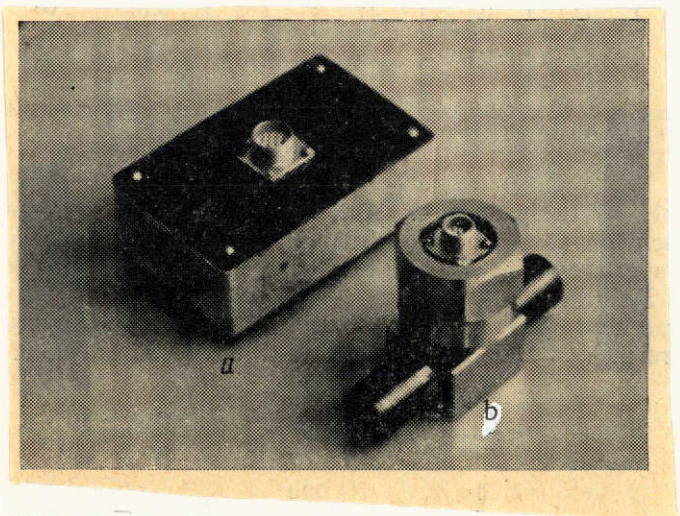
In virtue of its organoleptic and physico-chemical properties, regenerated water comes close to distilled water and does not meet the requirements for drinking water established by GOST [All-Union State Standard] 2876-54. Owing to the absence of soluble salts and gases, such water is unpleasant to the taste, does not quench the thirst, disturbs some physiologic functions of the organism and causes a number of illnesses [8]. To impart the requisite properties to regenerated water, it is enriched with mineral salts and trace elements. Experimentally demonstrated by this time has been the possibility of protracted use of regenerated and artifically mineralized water for drinking and domestic purposes and of setting up complete circulation of water in a closed ecologic system [7, 9].

In view of the fact that regenerated and mineralized water may contain pathogenic microorganisms, it becomes necessary to effectively disinfect it and stabilize its physico-chemical and hygienic indices during the preservation process. Our studies [4, 5] and those of S.V. Chizhov and his collaborators [2] have shown that of all the agents and preparations used for the preservation of drinking water, the most efficient are electrolytic solutions of silver (ESS). /47

Physico-chemical and microbiological studies have shown that ESS in doses of 0.1-0.2 mg/ℓ ensure a reliable bactericidal and preservative effect, and medico-biological studies, that these doses have no harmful effect on the human or animal organism [1, 3, 6]. Water processed with the indicated doses of silver keeps its high organoleptic and sanitary-hygienic indices for as long as a year or more [2, 4, 5].

Moreover, we studied the effect of the water's properties (color index, turbidity, chlorine ion content, total salt content, etc.) on the efficiency of preserving it with ESS. Thus, we established that the presence in water of chlorine ions (more than 120 mg/ℓ) from the suspended and organic substances to which the water's color index is due, considerably reduces silver's preservative effect. This points to the necessity of removing, to the fullest possible extent, suspensions and organic admixtures from the water incident to its regeneration, as well as to the necessity of minimally enriching the water with chlorine salts incident to its mineralization.

But in no more than a week after processing water with 0.1 mg/ℓ silver, these ingredients no longer have practically any telling effect, and even when the water was originally very contaminated with bacteria ($5 \cdot 10^6$ ind/ℓ), it becomes suitable for drinking, i.e. the preservative effect of silver depends considerably less on the indicated factors. Especially noteworthy is the fact that silver is a highly effective disinfecting agent with regard to the pathogenic microorganisms that cause acute intestinal infections, such as dysentery, typhus and cholera. It follows from the adduced data that the causative agents of acute intestinal infections are much less resistant to the action of silver than E. coli -- the principal sanitary index among microorganisms.



LKS-2 ionator: a. power package; b. electrolyzer.

Regenerated and mineralized drinking water can be disinfected and preserved aboard a spacecraft by ESS with the aid of a special LKS-2 ionator we developed. This device consists of a simple electrolyzer with silver electrodes (Sr. 999.9 silver) and a power pack that can be switched into the spacecraft DC electrical wiring system (see figure). The device can process 30 ℓ/hour regenerated and mineralized water with a silver dose of 0.1-0.2 mg/ℓ, maintained with an accuracy of ±20%. This dosing accuracy /48 is completely satisfactory, deviations being due to changes in the salt composition of the processed water and the concomitant variations in current silver output.

In the Institute of Colloidal Chemistry and the Chemistry of Water of the Ukrainian SSR Academy of Sciences we conducted

extensive tests with the LKS-2 ionator on a regenerated and mineralized water simulator with the following composition: alkalinity: 0.4-0.77 mg-eq/ℓ; hardness: 0.4-0.77 mg-eq/ℓ; fluorine ions: 1 mg/ℓ; oxidizability: 1.8 m/ℓ; pH: 7.1. The data in the table below show that the ionator worked normally after processing 1300 ℓ water, ensuring the prescribed dose of silver in the water. The silver electrodes of the LKS-2 ionator have enough resources to process 7500 ℓ water.

RESULTS OF TESTING THE LKS-2 IONATOR*

Quantity of processed water, ℓ	Electrode voltage, V	Water quality				Current silver output, %	Silver dosing accuracy, %
		Alkalinity, mg-eq/ℓ	Temperature, °C	Concentration, mg/ℓ			
				Fluorine	Silver		
30	12	0.6	22	0,2	0.17	85	+13
100	20	0,46	20	0,2	0.14	72	—7
176	20	0,52	20	1	0.13	65	—13
310	12	0,48	22	0	0,13	65	—13
460	22	0,4	18	1	0,15	77	±0
590	14	0,7	22	1	0.17	85	+13
630	14	0.7	21	1	0.13	65	—13
720	12	0.77	22	1	0.14	70	—7
810	17	0,71	18	1	0.16	80	+6
900	12	0.7	23	1	0.13	66	—13
990	16	0,74	18	1	0,13	66	—13
1300	17	0,7	23	1	0,12	60	—20

* Water discharge through electrolyzer: 30 ℓ/hour; strength of current passing through electrodes of ionator: 1.5 mA; calculated silver dose: 0.15 mg/ℓ.

In view of the fact that the ionator operates under pulse conditions when switched on for at most 1-30 sec per hour, we conducted appropriate tests

Pulse duration, sec	Silver dose, mg/ℓ, found by analysis*	Silver dosing accuracy, %
1	0,10	±0
3	0,09	—10
5	0.12	+20
10	0,10	±0
20	0,11	+10
30	0,09	—10

* Calculated silver dose: 0.1 mg/ℓ

The foregoing data indicate that electrolytic solutions of silver have a strong antimicrobial effect with regard to most microorganisms, including the causative agents of acute intestional infections that are transmitted through water.

In addition, inasmuch as it possesses the valuable property of preserving drinking water for a long time, water is indispensable under long-lasting space flight conditions. The LKS-2 ionator ensures the processing of regenerated drinking water by silver with a high accuracy under continuous as well as pulse conditions for a long time. Its light weight, size and power capacity permit it to be used in experiments on Earth as well as in space flights.

Abstract

The regenerated drinking water produced under spacecraft conditions may contain pathogenic microorganisms; it therefore becomes necessary to disinfect and preserve it effectively. It is shown that electrolytic solutions of silver have a strong antimicrobial effect with regard to most microorganisms, including the causative agents of acute intestinal infections; moreover, silver preserves water for a long time. The authors developed an LKS-2 device that ensures the processing of regenerated drinking water by silver with a high accuracy under continuous as well as pulse conditions. Nine references, one figure and four tables.

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USING BLUE-GREEN ALGAE IN CLOSED ECOLOGIC SYSTEMS

L. I. Rubenchik and V. A. Kordyum

A promising version of a closed system of circulating substances /49
aboard spacecraft is a system based on the vital activities of microorganisms, especially the unicellular green algae Chlorella, which is attracting a great deal of attention in connection with the problem of regenerating air, as well as water and food. Among the positive characteristics of Chlorella are the ease with which it can be cultivated, its rapid growth, and the formation of a profuse biomass. But as a potential component of a closed ecological system, Chlorella possesses a number of shortcomings:

1. Its mass cultivation requires the expenditure of energy on continuous stirring of the culture medium, which is necessary to create optimal conditions of photosynthesis and respiratory metabolism.

2. It is necessary to feed the culture with gaseous carbon dioxide; the amount of carbon dioxide that is contained in the air is not enough to yield a plentiful crop of this organism's cells.

3. To gather the cells it is necessary to use cell-precipitating substances, or to subject the liquid medium to centrifugation.

4. Chlorella belongs to the organisms that take up only bound nitrogen. It must therefore be completely provided with the appropriate nitrogen-containing compounds.

5. Under conditions of weightlessness, bubbling (the blowing of gas bubbles through the medium) is connected with great difficulties.

6. Separation of the biomass from the culture medium requires considerable expenditures of energy.

Inasmuch as the indicated shortcomings of Chlorella are irremovable, it is quite legitimate to look for other unicellular algae that might be free from these shortcomings. Among such organisms belong some representatives of the blue-green algae (Cyanophyta). In the present study we characterize the strain Anabaena variabilis 359, a nitrogen-fixing organism that was precipitated and obtained in an algologically and bacteriologically pure culture.

After preliminary testing of a number of media that have been recommended for the cultivation of blue-green algae, we opted for a Pratt medium, on the basis of which we studied the effect of different sources, as well as concentrations, of nitrogen and phosphorus on the growth and accumulation of a biomass of this organism. /50

The alga was cultivated in Erhlenmeyer flasks with a capacity of 100 and 250 ml containing up to 30 and 50 ml of nutrient medium, respectively. The culture was placed at 28-30°C on a rocker, above which there was a panel with luminescent lamps of the LB-30 type, with an illumination of 10,000 lx. The studies we conducted enabled us to find the optimal medium for growing and accumulating a biomass of the indicated alga when it takes up fixed nitrogen:

	mg/ℓ water
KNO_3	722
K_2HPO_4	28
$MgSO_4 \cdot 7H_2O$	50
$FeSO_4 \cdot 7H_2O$	20
Trilon B	16
$CaCO_3$	1500

The tracer element content per 1 ml of starting solution amounted to:

	mg
$Co(NO_3)_2 \cdot 6H_2O$	0.004
$ZnSO_4 \cdot 7H_2O$	0.008
HBO_3	0.280
$CuSO_4 \cdot 5H_2O$	0.002
$MnSO_4 \cdot 7H_2O$	0.200
$(NH_4)_6 Mo_7O_{24}$	0.100

The starting value of the medium's pH was equal to 7.0-7.5.

A crop of cells of strain 359 was spread out on the indicated medium and on the same medium without bound nitrogen. The crop on the nitrogenless medium turned out to be smaller: the daily growth of the dry biomass on 1 m^2 of illuminated surface amounted to 3.02 g as against 6 g when the alga took up nitrogen nitrate.

To compare growth in stationary and deep cultures the alga was cultivated in the indicated medium with bound nitrogen in flasks, some of which were set on the rocker (140 oscillations per minute) and some, on an immobile surface; illumination 350 lx; temperature 30°C. The crop in both series of flasks turned out to be practically the same (2.4 and 2.3 g/m^2 per day, respectively).

Experiments on the rocker showed that for the nitrogen-fixing algae, heterogenous growth is to be expected even under deep culture conditions. The biomass will be deposited on the walls of the cultivator in the form of foaming films or granules. We therefore used a cultivator in which the deposition of the biomass could be completely prevented by means of a gas-liquid separator (Fig. 1). Strain 359 was cultivated in such a cultivator in three media, with an identical basic salt composition, but the first contained bound nitrogen in the form of potassium nitrate, the second lacked potassium and the third lacked bound nitrogen.

In all three versions, a long lag phase could be observed, which usually lasted 10-14 days. The introduced culture underwent considerable changes. The inoculum was cultivated in the flasks on the roller. After they have been conveyed to the cultivators, the porous and diffuse microflakes turn into large, at first fine, and then dense, granules attaining diameters of up to 5-7 mm. The picture is different in the case of cultivating algae in a medium without potassium. In this case, the growth is more or less diffuse, differing but little from that on the rocker. The mean daily increments in the biomass depended mainly on whether or not bound nitrogen was present in the medium. Thus, a culture that grew under nitrogen fixation conditions yielded a mean daily increment of 3.72 g/m^2, while a culture that grew in the medium with nitrates and potassium, yielded 5.6 g/m^2. In the medium with bound nitrogen, but without potassium, the increment was approximately the same. /51

The amount of organic substance that the cells liberate into their surroundings also depended on the presence of bound nitrogen. When it was absent, the intracellular organic compound content amounted to approximately 1/3 of the cellular biomass, while in the media with the nitrate, it equalled the cellular biomass, and sometimes exceeded it. In the medium without bound nitrogen, the amount of nitrogen in the cells came to something less than 4%, while in the culture fluid, it varied from 8 to 21 mg/ℓ. When a culture grown in a medium with nitrates was introduced into a nitrogenless medium, the nitrogen content in the cells gradually dropped from 6.7% to a stable value of 3.7%. In all probability, the intracellular nitrogen enters into the composition of organic compounds inasmuch as it could not be detected by analyses for ammonia, nitrates or nitrites.

In cultivating blue-green algae in a fluid medium it is hardly expedient to refrain entirely from stirring the medium inasmuch as in so doing, stagnant zones are formed that can have a negative effect on growth. Therefore, we cultivated strain 359 under very restricted stirring conditions. Air (enriched with 3-5% carbon dioxide) was supplied and stirring was performed according to the pneumatic ram principle: air from the receiver, under pressure for a short time (tenths or hundredths of a second),

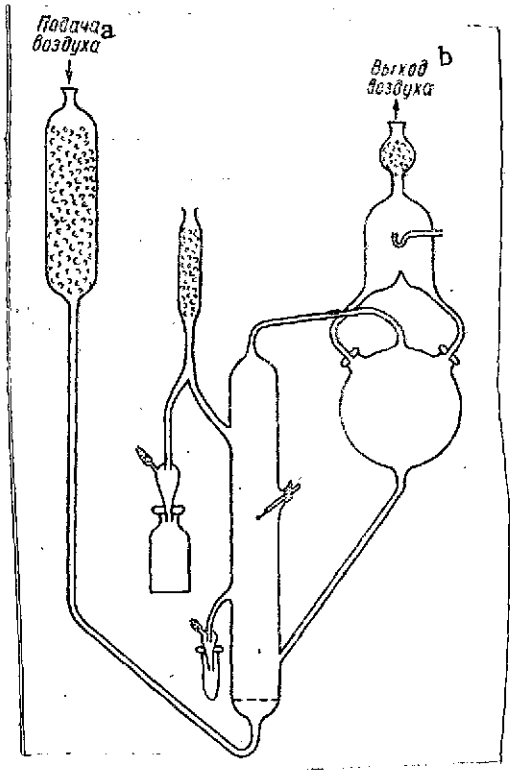

Fig. 1. Cultivator with washed antifoaming system that prevents the biomass from being deposited on the walls.

Key: a. Air inlet; b. Air outlet

entered the distributor -- a sphere with side arms -- whence it headed for the flasks in the form of a shock wave. As a result, air reached all the vessels and, by creating weak flocs (at the end of the path, the shock wave became attenuated), it ensured some stirring of the fluid.

For sterile cultivation we used special flasks with a continuous double section in the cork (Fig. 2). This design made it possible to sterilize the air through input filters and excluded the possibility of contamination from the outside inasmuch as the output filter prevented external air from penetrating, while the collar in the middle of the section did not allow contaminating cultures to germinate actively along the wetting surfaces. Growth in flasks with an pneumatic ram showed that limited stirring can ensure considerable crops, in some experiments up to 9 g/m^2 per day.

In studying blue-green algae we noted that these organisms grew better in solid nutrient media than in fluid ones. This served as the basis for working out a method of cultivating strain 359 in a two-component system including an agared nutrient medium and the membranous filter covering it (Fig. 3). Cultivation on the surface of the filter makes it easy to remove the crop and weigh it.

This means of cultivation was further developed, using for some filarial algae that do not fix nitrogen a cultivator (model No. 32) operating under irrigation conditions. A culture of algae that had been cultivated beforehand in a solid nutrient medium was deposited on a tiny glass net. From the pulverizer situated above the net, the deposited material, as well as the net itself, was continuously irrigated with fresh medium. Illuminating was effected downwards through a glass that prevented the sprayed fluid from dropping onto the illuminator. Experiments showed that it is possible to obtain on the net extremely high yields: up to 30 g from 1 m^2 per day. /52

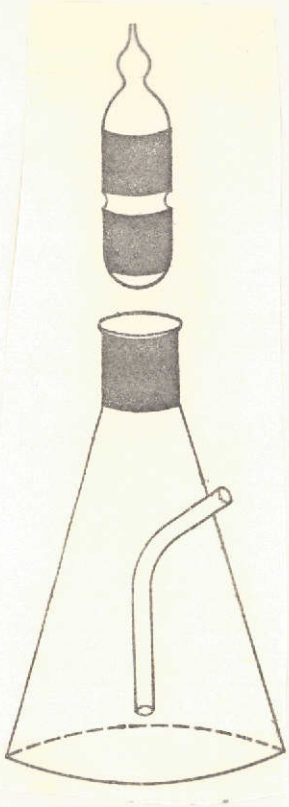

Fig. 2. Double-sectioned flask.

Besides drop irrigation of the growing culture, there exists another method of feeding the culture: by means of a solution of nutrient salts (as in the case of the aeroponics of the higher plants). We think that this method will turn out to be very compact and efficient, requiring much less energy to be expended. Aeroponics as applied to algae can solve the technically complicated problem of uniform distribution of the fluid over the entire surface of the growing culture. Besides, under conditions of weightlessness wetting on the basis of drop distribution is very difficult.

The cultivator of the "Aeroponics" system (Fig. 4) for cultivating the nitrogen-fixing strain 359 was based on the aeroponic principle. It differs from the above-described model No. 32 in that, instead of having the spraying cone fall directly onto the growing culture, the latter is irrigated by means of a reflected spray of water. Strain 359 was cultivated in this cultivator with an illumination of 10,000 lx. It has already been explained that it can grow even with a higher degree of illumination of the working surface -- up to 16,000 lx (with an illumination of 21,000 lx, the culture completely loses its color and dies). Under these conditions, we were able to obtain up to 20 g biomass on 1 m^2 per day under nitrogen-fixing conditions.

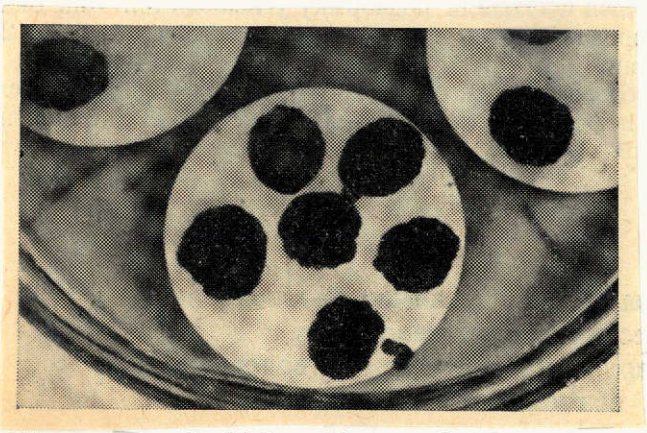

Fig. 3. Cultivation of blue-green algae on membranous filters.

The essential problem in closed ecological systems is nitrogen deficiency. In the process of mineralization, bound nitrogen is partially turned into molecular nitrogen, as a result of which a continuously decreasing amount of it will be found in the system. This can be eliminated in the case where the autotrophic culture of algae is simultaneously the nitrogen fixer, too, or if in the system, in addition to the autotrophes that do not fix nitrogen, there is a

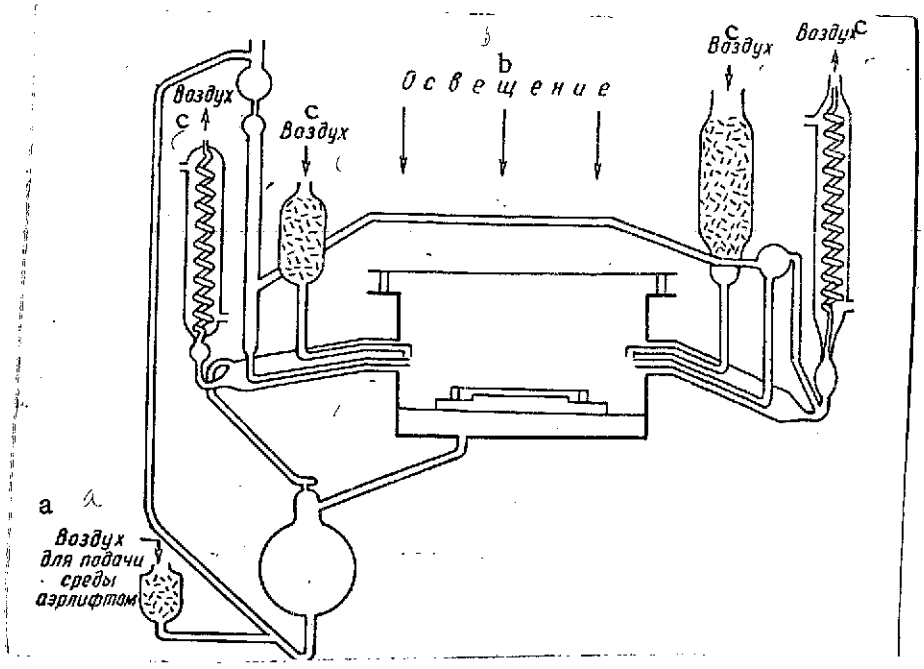

Fig. 4. System for cultivating blue-green algae under conditions of aeroponics.

Key: a. Air for supply to medium by air lift
b. Illumination
c. Air

connecting link with nitrogen-fixing ones, accumulating the same amount of nitrogen that it combined with during mineralization.

Besides feeding, nitrogen-bound systems, nitrogen-fixing algae have the advantage that on nitrogenless substrates, they can more successfully withstand their competitors -- "odd" algae. When growing under nitrogen-fixing conditions, algae generally contain less nitrogen compounds than do algae that do not fix nitrogen. Inasmuch as the problem of balanced feeding is acute in closed ecological systems and becomes a necessity in organisms in which the proportion of proteins, fats and carbohydrates might suit the requirements of man and animal, this reduction in the content of nitrogen components, especially protein, in the biomass of algae is very valuable. As our experiments have shown, Chlorella and Scenedesmus depress strain 359 when growing in the medium with bound nitrogen, whereas under conditions of nitrogen fixation, the latter prevailed in its competition with the other algae. When cultivated in the medium with bound nitrogen, strain /53
359 contained up to 40% protein, while in the medium without nitrogen compounds, it contained 20%, and sometimes even less.

As is known, the ability to fix atmospheric nitrogen is widespread among microorganisms, but in the majority of cases, this process is of low intensity. Only a few microorganisms are comparable, in their nitrogen-fixing activity, with Azotobacter or excel him, so the latter can serve as a convenient standard of comparison. Among microscopic organisms that bear comparison with Azotobacter, belong some strains of nitrogen-fixing blue-green algae of the genus Anabaena and Nostoc. These algae are widespread in nature, forming large colonies, are not fussy about their housing conditions and fix nitrogen relatively intensively.

To elucidate the nitrogen-fixing activity of strain 359 we took the above-mentioned medium, but without potassium nitrate. To this foundation medium we added potassium (in the form of 1.5 g chalk per 1 ℓ medium). Strain 359 was cultivated in 100 ml flasks at 30°C and an illumination of 11,000 lx. As the algae grew, the phosphorus was quickly depleted, so beginning with the sixth day after inoculation, we added phosphorus to the medium every 24 hours. Beginning with the third day, we carried out supplementary feeding with carbon dioxide every 3 hours. As it turned out, the alga fixed a very large amount of nitrogen -- up to 2.5 mg on 1 ℓ medium per day.

So we can conclude on the basis of our study that the prospects look good for blue-green algae as components of closed ecological systems.

Abstract

The advantages of blue-green photofixing algae over Chlorella are as follows: 1) it is not necessary to expend energy on stirring the culture fluid; 2) the crop can be gathered by a method that is simpler and more economical from the energy point of view; 3) there is no longer any need for bubbling to enrich the medium with carbon dioxide; 4) the molecular nitrogen of air can be used as nitrogen supply.

Selected for study is the nitrogen-fixing blue-green alga Anabaena variabilis strain 359, which belongs to the thermophils (temperature optimum 36-39°C); optimal illumination about 10 klx; maximal, 16 klx. The average crop in a many-month-long experiment comes to 4 g/m^2, soaring in a number of cases to 10 g/m^2. On conversion to the illumination that is appropriate for intensive Chlorella cultivation (70-100 klx), the average daily crop of the indicated strain of Anabaena is commensurate with that of Chlorella (40 g/m^2).

EFFECT OF DIFFERENT CONDITIONS OF NITROGEN SUPPLY ON THE CHEMICAL COMPOSITION OF SOME NITROGEN-FIXING BLUE-GREEN ALGAE IN CONNECTION WITH THE POSSIBILITY OF USING THEM IN CLOSED ECOLOGICAL SYSTEMS

L. V. Kosenko, M. Ya. Ratushnaya, V. S. Kirilova

Besides green algae, for example, Chlorella, blue-green algae can also be utilized to create closed ecological systems. /54 What is most likely to be used for this purpose is nitrogen-fixing blue-green algae. Some researchers [3, 26] regard these organisms as a potentially promising group of lower plants for air regeneration in closed ecological systems. It is supposed possible, with the aid of these algae, to return to the system the molecular nitrogen that is partially formed incident to mineralization of the organic nitrogen from products of the vital functions of biological objects. In addition, when closed ecological systems are being created, blue-green algae can be used as food. A number of authors [4, 18] indicate the use of some of them as food.

The purpose of the present study was to find highly productive strains of blue-green algae among the nitrogen fixers that are subject but little to the effect of changes in environmental conditions. We studied the effect of different conditions of nitrogen supply on the productivity and chemical composition of some nitrogen-fixing blue-green algae.

Methods and Materials. For investigation we chose 11 strains of nitrogen-fixing blue-green algae:

Anabaena variabilis Kütz. 359 & 550
A. oscillarioides Bory. 444
Amorphonostoc paludosum (Kütz.) Elenk. 446
A. punctiforme (Kütz.) Elenk. 500
Stratonostoc Linckia (Roth.) Elenk. 384 & 422
Sphaeronostoc coeruleum (Lyngb.) Elenk. 501
Pseudonostoc sp. 326
Calothrix Elenkinii Kossinsk. 489
Rivularia sp. 532

Two of these strains -- Anabaena variabilis 359 and Amorphonostoc paludosum 446 -- were studied in algologically, as well as bacteriologically, pure cultures; the other seven, in algologically pure cultures.

The algae were cultivated under optimal gas conditions [13] /55 in a mineral medium that had been selected beforehand. The crop

CHEMICAL COMPOSITION AND GROWING EFFICIENCY OF NITROGEN-FIXING ALGAE INCIDENT TO CULTIVATION UNDER DIFFERENT CONDITIONS OF NITROGEN SUPPLY

Group	Strain	Medium w/ nitrogen: Content in % of dry cell weight: Proteins	Carbohydrates	Lipids	Productivity of algae, g/l	Medium w/o nitrogen: Content in % of dry cell weight: Proteins	Carbohydrates	Lipids	Productivity of algae, g/l
I	*Anabaena variabilis* 550	34.3	30.0	4.3	0.47	31.6	31.0	4.0	0.46
	Amorphonostoc paludosum 446	26.4	27.2	3.8	1.15	24.4	26.6	6.0	1.14
	Pseudonostoc sp. 326	20.8	39.7	6.1	1.00	20.6	40.3	—	0.90
	Calothrix Elenkinii 489	23.5	25.4	3.3	0.59	24.8	30.4	5.9	0.64
	Rivularis sp. 332	28.3	28.2	3.0	—	28.1	27.7	—	—
II	*Anabaena variabilis* 359	37.5	26.0	7.2	0.57	24.6	30.0	13.0	0.40
	A. oscillarioides 444	23.5	23.8	4.4	0.21	20.2	34.5	—	0.11
	Stratonostoc Linckia 422	29.0	43.2	6.7	0.61	23.9	52.2	—	0.52
	Sphaeronostoc coeruleum 501	25.4	29.5	4.5	0.40	20.4	33.2	11.2	0.30
III	*Amorphonostoc punctiforme* 500	20.4	38.7	7.5	0.15	25.9	36.6	12.6	0.32
	Stratonostoc Linckia 384	24.6	29.9	7.6	0.43	31.6	29.4	7.0	0.54

was gathered after the culture had reached the second stationary stage of growth, i.e. on the ninth to fifteenth day of cultivation. The biomass of algae was separated from the culture medium by centrifugation; then, to determine the proteins and carbohydrates they were fixed in 96% alcohol and dried in a thermostat at 50°C. The alcohol residues were removed under a vacuum. To determine the lipids we used the damp biomass. The indices of biochemical composition that interested us were determined in the following manner:

-- the albuminous nitrogen in the cells of the algae, after the proteins had first been precipitated and extracted, by the Kjeldahl micromethod;

-- the total quantity of protein, by multiplying the albuminous nitrogen by the coefficient 6.25;

-- the carbohydrate content, colorimetrically by the anthrone method [27];

-- the lipids, by the weight method, after three-phase extraction with an alcohol-ether mixture and subsequent extraction of the lipids from this mixture by petroleum ether;

-- the dry weight, by drying a sample at 103-105°C to a constant weight. The productivity of the algae was computed on the basis of their content in grams in 1 ℓ culture medium. All determinations were repeated from two to six times.

Research results. Inasmuch as all the algae we investigated are nitrogen fixers [8, 9, 15], we were interested in finding out whether the chemical composition of their cells changes for different conditions of nitrogen supply. To this end, the algae were cultivated simultaneously in two versions: in a medium with bound nitrogen (KNO_3) and in one without nitrogen. As can be seen from the data of the above table showing the results of comparative analysis of the protein, carbohydrate and lipid content in the cells of algae cultivated under different conditions of nitrogen supply, proteins amount to 20.4-37.5% of the dry weight of cells incident to cultivation in a medium with nitrogen nitrate, and to 20.4-31.6% under conditions of nitrogen fixation; the carbohydrates, 26.0-43.2 and 26.6-52.2%, respectively; and the lipids, 3.0-7.6 and 6.0-13.0%, respectively. To judge from these indices, the algae we investigated differ but little from those studied by other authors, according to whom the cells of blue-green algae /56 contain 20-80% proteins, 6-75% carbohydrates and 2-12% lipids [1, 2, 10, 14, 16, 19, 20, 23-25].

The algae we studied evinced different abilities to grow under different conditions of nitrogen supply: in five strains, the productivity did not change either incident to cultivation in the medium with bound nitrogen or in that without nitrogen; in four, the productivity was higher in the medium with nitrogen, and in another 2, it was higher under conditions of nitrogen fixation. Here we can discern the following rule: in a medium conducive to its best growth, a culture contains more proteins and correspondingly less carbohydrates than in a medium in which it grew worse. Thus, on the basis of the productivity and the protein and carbohydrate content, the algae we studied can provisionally be divided into three groups:

I. Algae in which the growing efficiency is the same in both media: Anabaena variabilis 550, Amorphonostoc paludosum 446, Pseudonostoc sp. 326, Rivularia sp. 532. The protein and carbohydrate content in their cells does not depend on cultivation conditions. To this same group we can assign the strain Calothrix Elenkinii 489, in which the growing efficiency and cellular protein content is approximately the same in both media. The carbohydrate content in the cells in the medium with bound nitrogen is somewhat less (25.4%) than in that without nitrogen (30.4%); if, however, we take into account not only the cellular carbohydrates, but also the carbohydrates liberated in the culture medium, then their total content depends but little on the medium: 29.0% in the medium with bound nitrogen, and 32.0% in the medium without nitrogen.

II. algae in which the growing efficiency is greater (by 0.10-0.17 g/ℓ, depending on the type of alga) in the medium with bound nitrogen than under conditions of nitrogen fixation: Anabaena variabilis 359, Anabaena oscillarioides 444, Stratonostoc Linckia 422, Sphaeronostoc coeruleum 501. In this case the protein content in the medium with bound nitrogen was greater (by 3-13%0), and the carbohydrate content, less (by 3-11%), than in the medium without nitrogen.

III. algae in which, on the contrary, the growing efficiency is less (by 0.11-0.17 g/ℓ) in the medium with bound nitrogen than in the medium without nitrogen: Amorphonostoc punctiforme 500 and Stratonostoc Linckia 384. In this case, the protein content in the medium with bound nitrogen was less by 6% than in the medium without nitrogen. Cultivation conditions had almost no effect on the cellular carbohydrate content, but the total cellular and extracellular carbohydrate content in the medium with bound nitrogen was greater by 8-15% than in the medium without nitrogen.

Thus, when the algae we studied are cultivated under different conditions of nitrogen supply, what changes most of all is their productivity. The different degree of adaptation of blue-green algae to certain nitrogen sources has been shown by a number of other authors. According to the data of V.P. Pomiluyko [11, 12], incident to low concentrations of bound nitrogen in, or its exclusion from, the medium, the photosynthetic activity of algae of the genus Microcystis is depressed. This author has also shown that nitrogen sources (urea, nitrates and nitrites) influence the tempos of reproduction of blue-green algae, but that the relation of the latter to nitrogen is clearly characterized by specificity. On the other hand, the nitrogen of the medium may inhibit growth and nitrogen fixation. According to the data of Zh. P. Kopteva [7], the presence of "starting" nitrogen in the medium inhibited the process of nitrogen fixation in Anabaena variabilis, Nostoc punctiforme, Nostoc sp. and other species of algae; in this case, the increment in the biomass, too, was reduced, sometimes very considerably. In our experiments, the worse growth in one medium as compared with another may possibly be connected with the fact that some conditions (light, temperature and others, which are necessary for optimal productivity and nitrogen fixation), may differ for cultivation in a medium with bound nitrogen and for conditions of nitrogen fixation, as this has been shown by Feoktistova [17] for Stratonostoc Linckia in relation to temperature.

The differences in the protein and carbohydrate content observed for different growth of the algae in the media utilized is probably connected with a change in the intensity of their growth processes. A number of data in the literature support this conjecture. In particular, it has been shown [5, 6, 21, 22] for green algae that under those conditions which are optimal for the growth of the culture, there is an intensified breeding of progeny, /57

and the synthetic processes of the organism are directed toward the creation of substances, above all albuminous compounds, that are necessary for the formation of daughter cells. When the growth of the algae is retarded, protein synthesis is also retarded, and the main products of photosynthesis can be employed by the organism to form reserve material — carbohydrates and lipids.

Thus, we could detect no direct dependence of the protein and carbohydrate content on conditions of nitrogen supply. The conditions of nitrogen supply did have an effect on the productivity of the algae and, in connection with the different intensities of the processes incident to growth in one or another medium, on the accumulation of proteins or carbohydrates: in the medium conducive to the best growth, algae form a greater quantity of albuminous substances and a lesser quantity of carbohydrates; under the worst growing conditions, there is a greater accumulation of carbohydrates and a lesser accumulation of proteins. At the same time, no markedly pronounced dependence of the accumulation of lipids on the growing efficiency of the algae could be detected. A higher (by a factor of 1.5-1.8) lipid content has been established for the cultivation of blue-green algae under conditions of nitrogen fixation as compared with algae cultivated in a medium with bound nitrogen.

The strains of nitrogen-fixing blue-green algae that we studied are characterized by a certain resistance of the metabolic processes, which is borne out by the small difference between the mean indices for algae cultivated under different conditions of nitrogen supply. Taking into account the fact that the algae we studied are good nitrogen fixers, the data we obtained allow them, in particular Anabaena variabilis 550, Amorphonostoc paludosum 446, Pseudonostoc sp. 326, Rivularia sp. 532, to be regarded as potential objects in choosing algae for closed ecological systems.

Abstract

The different abilities of nitrogen-fixing blue-green algae (in all, 11 strains) to grow incident to cultivation under different conditions of nitrogen supply have been established. The relation between the growing efficiency of the algae and their protein and carbohydrate content has been shown: in a medium that is conducive to their best growth, the algae form a greater quantity of albuminous substances and a lesser quantity of carbohydrates. Under their worst growing conditions, more carbohydrates and less proteins are accumulated. The lipid content is 1.5-1.8 times higher incident to cultivation of the algae under conditions of nitrogen fixation. Twenty-seven references and one table.

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ON THE POSSIBILITY OF CREATING SYSTEMS FOR THE RAPID PREDICTION OF THE STATE OF CHLORELLA POPULATIONS ON THE BASIS OF DYING-OFF CURVES

V. A. Kordyum

In working with algal cultures, it often becomes necessary to know something about the state of the population [1]. One of the characteristics of this state is the live-to-dead cell ratio [2]. At the same time, while a considerable number of methods have been proposed for determining dead cells in bacteria, in the case of algae, there are practically no such methods.

A differentiation of cells into dead and live can be based on a number of principles. Most often, the total number of microbes is compared with the number of organisms that are capable of germinating. The latter are determined either by dish seeding or by microscopic determination of the cells that are starting to grow [16]. Another set of methods is based on pigmentation peculiarities of the dead cells. Differentiation by these methods is based on the rapid breakdown of nuclear material incident to dying away or by impaired permeability. In the first case, the live and dead cells differ in color when stained [7]; in the second, the live cells lose their color, while the dead cells are stained. In the latter case, either ordinary or luminescent dyes can be used. It is easier and simpler to use ordinary dyes: no special optics are required. Luminescence microscopy with fluorochromes is more complicated but allows up to 1-2 orders less dye to be used [2, 8, 9].

For some organisms, in particular algae, killed cells can be determined by the dying out of luminescence in the ultraviolet that is connected with chlorophyll destruction. Finally, in some studies, live cells were distinguished from dead by the restoration of the former by tetrazole salts in formazan [3, 17]. This profusion of methods and principles explains the absence of a universally accepted method for all organisms [9].

It would seem simplest of all, in the case of algae, to use chlorophyll's property of natural luminescence. But chlorophyll destruction after the dying-off of the zooid may stretch out in time, and a blurred response will be obtained. Besides, required in this case, just as in the case of using fluorochromes, is a luminescence microscope, which is not always on hand. If, on the other hand, luminchromes are used, another complication crops up: the cells are killed by UV radiation, in which case they become colored. This entails the danger of overestimating the actual percentage of dead cells. As far as tetrazole is concerned, it does not reveal all live cells [9].

Theoretical analysis and preliminary experimental observations have shown that simple to use and most expedient for differential staining are nonluminescent dyes, preferably primary fuchsin and methylene blue [5, 6]. To determine the results of staining, we prepared a mixture of live and dead cells in a known proportion. Comparison of the calculated value with that obtained experimentally permitted the quality of the differential staining to be judged. The staining itself was performed by mixing equal volumes of culture suspension and dye. The latter was taken in such a concentration as to ensure its prescribed dilution after mixing. /59

Another virtue of the recommended method is the rapidity of staining: a specimen was completely stained in the very process of preparation. It is also maximally simple to prepare the specimen according to the crushed drop principle, and the time spent on its preparation is minimal. Differential staining should be performed with due regard for the fact that the dyes must be prepared on a low-salt basis (Pratt medium type), without phenol, whereupon the reagent is suitable for use only on the day of its preparation.

Being simple and efficient, differential staining of live and dead Chlorella cells with the aid of primary fuchsin or methylene blue can be recommended for laboratory practice. But differential staining does not allow the physiological state of the culture to be judged. An answer to this question can be obtained by using other reagents. Thus, in the last decade tetrazole salts have become prevalent. Penetrating into the cell, they are restored with formation of a stained compound -- formazan -- that is insoluble in water.

Inasmuch as the centers of oxidation-reduction reactions in the cell are the mitochondria, tetrazole salts proved to be convenient reagents for staining them [1]. Moreover, by setting up different conditions with the aid of these compounds, we can detect and differentiate a number of fermentation systems in animal, plant and microbe cells [7, 12, 16]. Considerably less well studied has been the possibility of using tetrazole in the study of unicellular algae. But even in those few studies that are devoted to individual representatives of the indicated group of organisms, only 2,3,5-triphenyltetrazoichloride was used [4, 15]. We were unable to stain Chlorella cells with this tetrazole.

With reference to methods of staining with tetrazole salts, contradictory reports are found in the literature. In spite of the fact that, in accordance with the latter's mechanism of action, anaerobic conditions are necessary for staining, in some cases good results were obtained without the admission of molecular oxygen [14] and in others, in its presence [10].

We therefore conducted research under different staining conditions:

-- with the admission of oxygen, but without stirring in scattered light;

-- with the admission of oxygen, but without stirring in the dark;

-- with the admission of oxygen, but without stirring in the dark with an additional 0.1% glucose as energy material;

-- under anaerobic conditions in the dark with the addition of 0.1% glucose as energy material.

Anaerobosis was achieved by blowing nitrogen through the mixture of tetrazole solutions with the algal suspension and subsequent hermetic sealing of the test tubes.

Starting from the data in the literature, for our first test we took six samples of tetrazole in concentrations of 0.05 and 0.01%. A positive result was obtained in all cases only with nitroblue tetrazole (both specimens) and INT[1]. But INT stained a small percentage of the cells and yielded very unstable results. Staining with nitroblue tetrazole was more stable. Tests showed that the specimens stained better in the light incident to admission of atmospheric oxygen, but not incident to anaerobiosis in the dark.

Determination of the percentage of live cells in the population and their physiological activity permits a better understanding of the state of the culture. But the information obtained with customary staining methods is far from complete inasmuch as it is possible to show only extreme states -- life or death -- of the members of the population. Moreover, live cells differ from one another in their physiologic activity, potential viability and a number of other indices. To determine these differences by customary methods is complicated, and sometimes even quite impossible. Staining is the simplest means of bringing out the internal characteristics of cells, and we are keen to possess a staining system that would permit different aspects of the functional state of the cells to be determined and the state of the population as a whole to be judged on the basis of this information. /60

Each method has its own characteristic resolution. Differential staining is determined by the range of changes that it is still possible to detect by tinctorial differences. In the above example of such staining, the resolution is very low inasmuch as

[1] A brand of tetrazole.

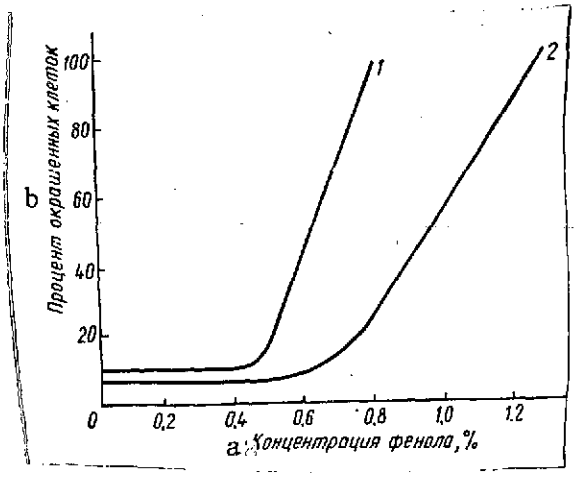

Fig. 1. Dying-off curve of starting and preheated cultures: 1. culture preheated for 1 hour at 55°C; 2. control culture.

Key: a. Phenol concentration, %: b. % stained cells.

not all transitional states between live and dead cells are taken into account. In principle, however, the sensitivity of the method can be enhanced. For all their differences, the properties of the cells constituting a population have one trait in common -- they influence cell viability. It is precisely in terms of viability that the phenotypic differences among members of a population can be brought down to a common denominator [11].

By "viability" we mean the ability to withstand unfavorable factors from the external environment. From this definition ensues a practical method of determining the viability and, consequently, the heterogeneity as well, of a population. With increasing concentration (or acting time) of some abiotic factor, the number of dead cells will increase, on the basis of which we can plot an appropriate distribution curve to characterize the given population. The dying-off of cells against a toxic agent background is a first step in enhancing the sensitivity of the tinctorial method of determining states of the organism. These dying-off curves characterize the potential ability of the population.

After testing a number of agents, we opted for phenol. Its time of action on the cells was limited to 5 min of exposure. After some training, distribution curves can be plotted in less than an hour. Distribution of cells according to their viability permits very sensitive control over the mass culture. It has been established experimentally that the dying-off curves for one and the same cultivator, plotted at different times, differ from one another, which points to variations in the cells' "reserves of strength." Within certain limits, such variations are not dangerous, but reduction in viability is a warning that some negative factor is putting in an appearance. Of course, about the nature of the latter nothing can be said on the basis of dying-off curves; but the very fact of their early detection deserves attention.

On dying-off curves can be based a method of rapid determination of the resistance of a culture to the action of some factor, for example, temperature. Within certain limits, temperature variations do not have any marked consequences. A drop in /61

temperature leads to a slowing down of growth, but has no substantial effect on the cells' viability -- with a subsequent rise in temperature, they quickly return to their initial state. Quite another state of affairs emerges incident to a rise in temperature: at first viability drops off, and then the culture dies. This dying is easy to note. Considerably more complex to detect are the early stages of disturbance.

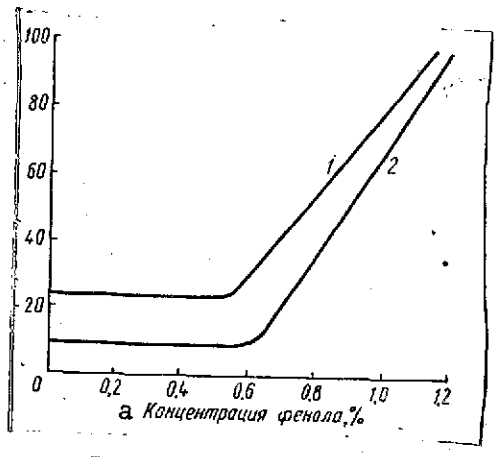

Fig. 2. Effect of gravitational extreme on Chlorella: 1. culture centrifuged for 5 min at 3000 rpm; 2. control culture.

Key: a. Phenol concentration, %; b. % stained cells.

We worked with the thermophil strain Chlorella vulgaris 62/5. The culture was heated at different times and temperatures. Then the dying-off curve was plotted against a phenol background. Two stages of aggravation were obtained. Latently different cells react differently to a temperature shock, which, by turning this latency of the cells into potency, caused, in the first place, a general decrease in the culture and, in the second place, intensified the heterogeneity of the population. Phenol transformed the consequences of the temperature shock into dead cells, once more enhancing the resolution of differential staining. As a result, despite the coincidence, in a number of cases, of the initial points before and after heating, different dying-off curves were obtained. The higher the temperature and the longer its action, the more strongly the population's viability dropped off (Fig. 1). Heat resistance is subject to the same variations as the content of live and dead cells at different sampling times.

We investigated, too, the population's resistance to ammonia. Temperature and ammonia in a certain range of values are very harmful agents. We were interested to check the sensitivity of the method to very mild effects. We opted for the centrifugation model, about whose mildly traumatizing effect there are clear indications in the literature. The dying-off curves plotted before and after centrifugation showed some decrease in resistance to the background agent after centrifugation (Fig. 2).

In view of the very high sensitivity of this method of plotting dying-off curves, we may assume that the given method of population testing will turn out to be useful in regulating the behavior of closed ecological systems.

Abstract

To determine the potential viability of microorganism cells, a method involving dying-off curves is proposed. Its possibilities are demonstrated on the example of the unicellular green alga Chlorella. The essence of the method is to determine the number of dead cells against the background of a nonspecific toxic agent. THe curve of percentage of dead cells versus dose of nonspecific toxic agent characterizes the state of the population at a given moment and gives grounds for predicting its future behavior.

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COMPARATIVE STUDY OF RESPIRATION, PHOTOSYNTHESIS, PIGMENT FORMATION AND ULTRASTRUCTURE IN DIFFERENT CHLORELLA STRAINS

V. A. Kordyum, L. O. Eynor, Ye. K. Ostapenko, and L. V. Polivoda

The unicellular grean alga Chlorella is a convenient subject /62 for different model experiments. The existence of a rich variety of strains, the simplicity of cultivation and the inherent advantages of a model subject make it possible to carry out a wide range of comparative studies. Pigmentation mutants are especially convenient. No possible change in such vital functions as respiration and photosynthesis can go as far as complete suppression of the process -- otherwise, a lethal mutation develops. The range of variability can be increased by using a selection of mutational variants which, completely bereft of their photosynthesis capability, grow in organic nutrient media. But a change in function does not imply a loss of the cell's sensitivity to the inducing factor. Therefore, it also appears possible to explain the range of inducibility in organisms with impaired production of the substances that are controlled by the given inductor. Such studies are very current in the case of the protocariots (bacteria) [1, 3, 9, 15, 29, 32, 36]; extremely few [13, 21] such studies have been undertaken on the eucariots.

The latter circumstance is due exclusively to the absence of suitable subjects. In our opinion, as such subjects can serve the pigmentational mutants of Chlorella, in which the inducible sign will be a change in pigment content under the action of light.

The purpose of the present study was to explore the possibility of using Chlorella as a simple and convenient model for studying the distinctive features of inducibility in organisms with a differentiated nucleus. In spite of the great number of studies of the different characteristics of a number of individual strains of Chlorella [16, 19, 25, 28], almost no complex studies [26] have been simultaneously undertaken of a considerable number of indices in general and in connection with photoinduction.

We measured O_2 liberation and absorption by different mutants of Chlorella in the dark and in the light in mineral and organic nutrient media and studied the change in pigmentation characteristics in the process of photoinduction, as well as ultrastructural peculiarities of the cell.

Methods and materials. We studied the rich thermophil strain Chlorella vulgaris 62 (s. 62) [6] and 14 mutational strains of Chlorella. The pigmentational mutants of Chlorella

L_1, L_2, ss. 14 were obtained, as was ss. 62, in the Department of General and Soil Microbiology of the Microbiology and Virusology of the Ukrainian SSR Academy of Sciences [7]; the mutants B-8, g-9, g-14, g-33, g-34, w-5w, w-5g, A-30-1 and 43-13 [5, 14] were kindly placed at our disposal by the Biology Institute attached to LGU [Leningrad State University?]; the mutant strains T5E10 and T5E11 were obtained from the institute of General Genetics of the USSR Academy Sciences (Moscow). The mutant clones were stable. In spite of the fact that they had been cultivated in laboratories for several years, control tests never once disclosed throwbacks in them. Nor could the appearance of any considerable number of pigmentational isomutants be recorded in the basic population. /63

In the experiments, we used only bacteriologically pure cultures. The algae were incubated in the dark for 3 days at a temperature of +34°C in a solid FDAGA [4] nutrient medium. Immediately before the experiment, the biomass of algae was washed off from part of the agar swarms with fluid FDAGA medium (organic medium), and from the other part, with a medium of the same composition, but prepared without glucose or a yeast autolyzer (mineral medium). For the latter version, before the experiment the algae were washed off with mineral medium. The density of the algal suspension was usually brought up to 50-70 million cells per 1 ml.

Breathing and photosynthesis were studied by the manometric method.

We studied the oxygen metabolism of the algae in the light and in the dark. The CO_2 content in an air atmosphere in 20 ml vessels was kept at the 1% level with the aid of a Pardi buffer [10]. We introduced 2 ml Chlorella suspension into a medium of appropriate composition. The experiments were repeated three times, using 2-3 vessels in each experiment. Illumination at the vessel level was about 15,000 lx (DRL-750 lamp). In our experiments, we used the "manometric instrument" model for studying the photosynthesis and respiration of plants [11] with a high degree of heat resistance (deviations from the given temperature were within the limits of 0.02°), independently of lighting conditions. The temperature in the thermostatic bath was kept at +34°C, and the contents of the test samples were continuously stirred. The frequency of the vessels' to-and-fro movements amounted to 2 Hz. The rest of our working procedures did not differ from the generally accepted [10, 12]. Changes in the partial pressures in the manometric instrument were measured every 30 min, but all calculations were carried out for 1 hour and on 100 million cells per 1 ml. The exposure times of the algal suspensions in the dark and in the light amounted to 1-1.5 hours in different experiments.

In parallel with the study of the change in the processes of oxygen metabolism under the action of light, we investigated the

pigmentational characteristics of s. 62 and the pigmentational mutants L_2, g-9, g-14, g-33, B-8, w-5g, A-30-1, T5E10 and T5E11 before and after illumination. The absorption spectra of the investigated cultures were recorded before and after incubating them in the light for 2 hours under the above-mentioned conditions within the limits of 400-750 nm with the aid of an SF-10 self-recording spectrophotometer. To characterize more clearly the change in the absorption spectra of the suspensions during 2 hours of cell illumination, their difference spectra were recorded (suspensions of illuminated cells versus an equal suspension of cells kept in the dark at the same temperature). In the latter case, we used a new photometric checking device whose full revolution on the scale of changes in optical density was equal to approximately 0.1D (and not 2.5D, as in the SF-10 factory checking device). The scale of resolution was therefore increased 30-fold, whereby we were enabled to get a clearer recording of the change in the absorption spectra of suspensions of cells of different strains in the course of 2 hours of illumination.[1] For electron microscopic analysis, the investigated material was subjected to centrifugation (4000 g) and fixed with a 1.2% solution of $KMnO_4$ with subsequent prefixation in a 2% solution of OsO_4. The Chlorella cells were dehydrated by passing them through a series of alcohols, after which the material was drenched in a mixture of methacrylates. Cuts were made with a Swedish ultratome; they were contrasted by lead nitrate. Microphotographs were obtained with the aid of a UEMV-100V electron microscope.

Results and discussion. In the first stage of the investigation, we recorded the general characteristics of Chlorella cultures. On the basis of the data about the change in the oxygen metabolism under the action of light and in the absence of illumination that are shown in the table, the investigated normal and mutant strains of Chlorella can be divided into the following groups in terms of the reaction of their oxygen metabolism to light and the degree of inertia of the changing over of their metabolic processes under the action of light and dark: I. photoneutral; II. inertia-photosynthesizing and inertialess-breathing; III. inertia-photosynthesizing and inertia-breathing; IV. photoinertialess and inertia-breathing; V. inertialess. /64

To group I belong strains L_1, L_2, g-14, g-34, w-5w, w-5g, 43-13 and T5E11, in which O_2 is absorbed more or less uniformly, independently of any change in lighting conditions.

S. 14, a representative of group II, reacts to illumination following a dark phase by a reduction in O_2 absorption, this property appearing much more clearly incident to a second

[1] The SF-10 instrument was modified by L.O. Eynor and V.I. Ryabtsev on the recommendation of co-workers from the Moscow State University [?]!

RESPIRATORY METABOLISM IN mkl O_2 PER 100 MILLION CELLS CALCULATED PER 1 HOUR

Strain	Dark			Light			Dark		Light
	1 hr	2 hr	3 hr	1 hr	2 hr	3 hr	1 hr	2 hr	3 hr
Mineral medium									
L_1	—22.5	—49.5	—40.0	—40.0	—42.5	—40.0	—34.0	—29.0	—39.0
L_2	—70.5	—63.0	—54.5	—60.0	—72.0	—73.5	—65.0	—53.0	—60.0
g-14	—40.0	—45.0	—62.0	—62.0	—56.0	—50.0	—55.0	—47.0	—45.0
g-34	—50.0	—50.0	—40.0	—45.0	—27.0	—30.0	—25.0	—31.0	—25.0
w-5g	—79.0	—68.0	—65.0	—62.5	—51.0	—40.0	—56.5	—42.0	—45.0
w-5w	—64.0	—78.0	—106.0	—106.0	—124.0	—100.0	—132.0	—110.0	—102.0
43-13	—45.5	—37.5	—55.0	—47.5	—71.0	—67.0	—89.5	—70.5	—56.5
T5E11	—100.0	—125.0	—92.5	—195.0	—171.0	—175.0	—220.0	—255.0	—93.0
14	—30.0	—42.0	—45.0	—37.5	—31.5	—22.5	—55.0	—46.0	—10.0
B-8	—	—35.0	—40.0	—40.0	—33.0	—25.5	—26.0	—24.5	—24.5
g-9	—17.5	—23.0	—18.0	—14.0	—14.0	—8.3	—13.0	—12.5	—11.0
g-33	—18.0	—18.0	—15.0	—18.0	—12.5	—12.0	—11.0	— 8.5	—8.5
A-30-1	—80.0	—98.0	—106.0	—88.0	—41.5	+25.0	—68.0	—68.0	+126.0
T5E10	—92.0	—148.0	—161.0	—128.5	—82.5	—52.5	—68.0	—38.5	—57.0
62	—44.0	—68.0	—54.0	+125.0	+145.0	+188.0	+ 0.7	—38.0	—
Organic medium									
L_1	—20.0	—39.0	—40.5	—45.5	—45.0	—49.5	—43.5	—40.5	—49.5
L_2	—31.5	—47.5	—48.0	—49.0	—56.5	—63.0	—52.5	—50.0	—45.0
g-14	—44.0	—58.0	—66.0	—82.0	—70.0	—58.0	—38.0	—55.0	—58.0
g-34	—111.0	—137.5	—125.0	—132.5	—73.5	—73.5	—62.0	—75.0	—56.0
w-5g	—70.0	—95.0	—56.5	—94.5	—87.5	—57.5	—75.0	—48.0	—43.5
w-5w	—59.0	—84.0	—92.0	—94.0	—113.0	—96.0	—130.0	—126.0	—90.0
43-13	—91.0	—88.0	—91.0	—119.5	—109.0	—118.0	—115.0	—119.5	—108.0
T5E11	—139.0	—116.5	—125.5	—181.5	—205.5	—176.0	—261.0	—322.0	—83.0
14	—39.0	—43.0	—58.0	—58.5	—50.0	—41.0	—75.0	—70.0	—38.0
B-8	—	—37.0	—51.0	—54.5	—49.0	—49.5	—44.5	—38.0	—48.0
g-9	—22.0	—28.5	—27.0	—26.0	—23.0	—17.0	—23.5	—27.0	—18.0
g-33	—38.5	—51.5	—49.5	—52.5	—35.5	—23.0	—26.0	—36.5	—25.0
A-30-1	—22.5	—53.5	—57.0	—50.0	—28.0	—16.7	—68.0	—65.5	+10.0
T5E10	92.5	—139.0	—162.0	—151.0	—122.5	—80.5	—85.0	—38.5	—38.5
62	—47.0	—48.0	—48.0	—28.5	—11.0	—25.5	—61.5	—65.5	—

illumination. Incident to shading the cells of s. 14, a high level of O_2 absorption is recorded at once, whereupon the former illumination at first even intensifies this process. Consequently, photoinduction does not lead to such a reorganization of the metabolism of this strain as might require a return to the initial state incident to subsequent shadowing.

In s. g-9 and g-33, which are representative of group III, O_2 absorption gradually decreases as the cells are transferred from the dark to the light and increases incident to darkening. The time lag of the breathing and photosynthesis processes is very high.

The photoinertialess and inertia-breathing s. 62, which belongs to group IV, is characterized by a rapid transition to photosynthesis conditions incident to lighting. Incident to /65

shading, however, the level of O_2 consumption is restored very slowly. We may assume that such a reaction to shading is connected with the necessity of sufficiently protracted reconstruction of the metabolic processes, as a result of which the cells undergo a state of total equilibrium of O_2 liberation and absorption for the measured periods of time.

Strain A-30-1 is a representative of the group including inertialess cultures, i.e., such as go over practically at once to the gas phase corresponding to illumination. After being transferred to the light, they photosynthesize, and after shading, they begin to consume O_2 at once.

Thus the analysis we carried out permits Chlorella strains to be divided into a number of groups on the basis of their degree of inducibility. We were interested to compare the representatives of these groups in terms of the photoinduction of some other criterion. Therefore, in addition to the respiratory metabolism, we investigated the pigmentational characteristics of the same Chlorella strains. In representatives of group I (photoneutral), under the action of light a considerable change in absorption can be observed only in the range from 400 to 500 nm. Green pigments either are detected neither in the dark nor in the light (Fig. 1, a) or are formed in the dark in an insignificant number without change or even with some tendency toward a decrease under the action of light (Fig. 1, b).

The pigmentational set of s. 14 (Fig. 1, c) -- a representative of group II -- is characterized by the presence of a rather great number of yellow pigments and the absence of green ones incident to the cultivation of the algal culture in the dark. After light induction, however, a peak appears in the range from 660 to 680 nm, which corresponds to the chlorophyll which has made its appearance, and the peaks that are characteristic of the yellow pigments increase, which corresponds to the data on the photoinduction of the oxygen metabolism.

The pigmentational set of s. g-9 (Fig. 1, d) -- a representative of group III -- changes after 2 hours of incubation in the light in the direction of an increase in the peaks that are characteristic of chlorophyll and yellow pigments, in parallel with a change in the staining of the algal suspension from yellow to green. In general, this agrees with the data obtained when studying the respiratory metabolism of the given mutant. Noteworthy is a clear lag in s. g-9 as compared with s. 62, of the transition to photosynthesis as compared with the level of pigment biosynthesis (Fig. 1, d, e). We may therefore expect that group III consists of two subgroups: characteristic of the first is slow pigment induction and, as a consequence, a lag in photosynthesis, while characteristic of the second, as can be seen on the example of mutant g-9, is a delay in the activation of

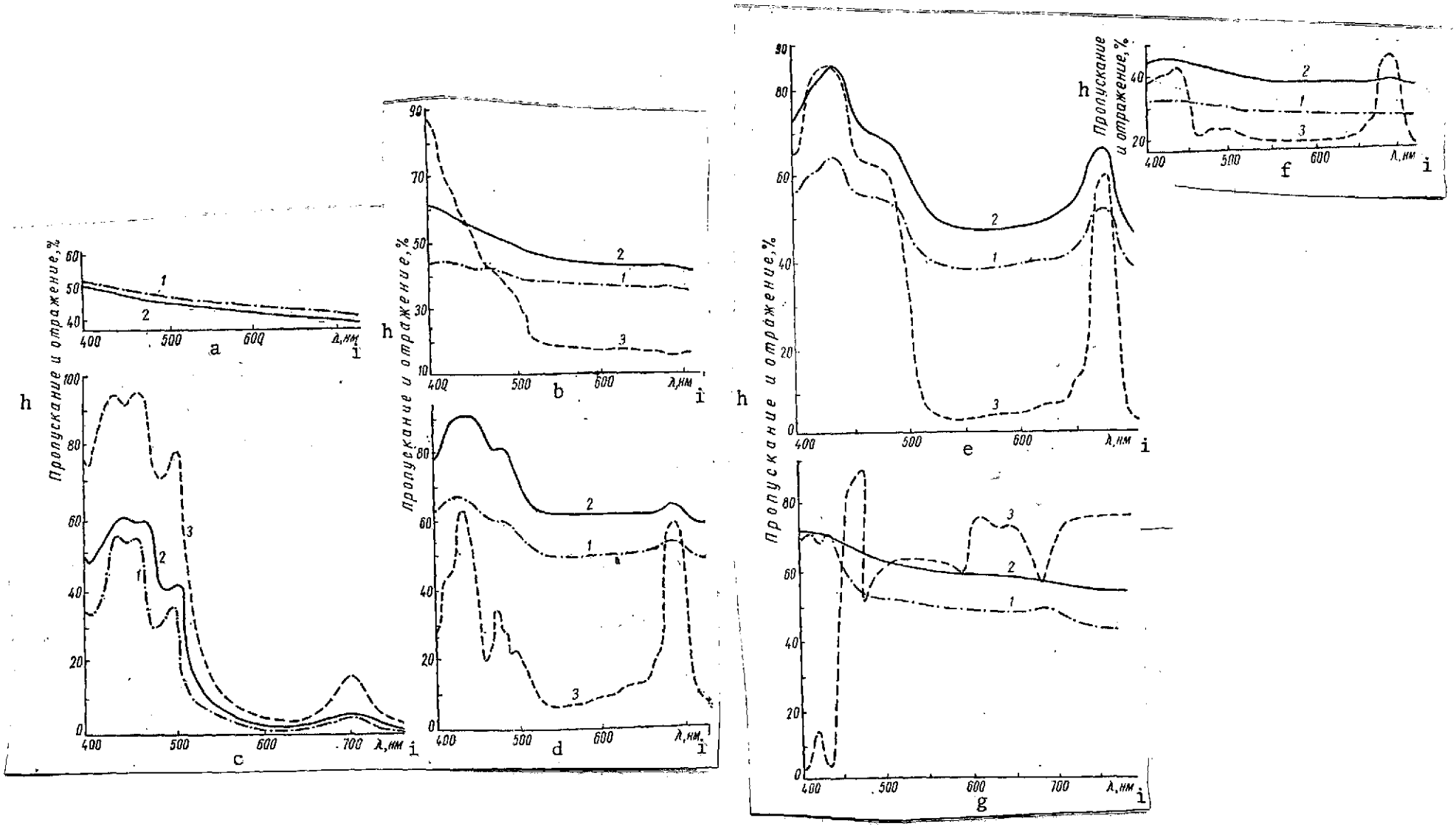

Fig. 1. Change in pigment composition of different Chlorella strains as a result of photoinduction. Strains: a. g-14; b. L_2; c. 14; d. g-9, e. 62; f. A=30-1; g. g-33; 1. dark control; 2. spectrum of Chlorella after light induction; 3. differential spectrum.

Key: h. Transmission and reflection, %; i. nm

those stages of photosynthesis which cannot be reduced to pigment formation. We can surmise that in them, control is disturbed in the process of photoinduction of pigment biosynthesis.

Fig. 1, e shows the absorption spectrum of s. 62 -- a representative of group IV; this spectrum is typical of phototropic algae. The peaks in the region of carotenoid absorption correspond to 472-482 nm. The absorption peaks in the 660-680 nm region correspond completely with the presence of chlorophylls a and b. The differential spectrum recorded from s. 62 after 2 hours of incubation in the light points to a considerable increase in absorption in the zone that is characteristic of chlorophylls and yellow pigments, as compared with the corresponding values for the variant that was incubated in the dark. This agrees with the data we obtained when studying the gaseous metabolism of the indicated strain and indicates that for it, light is an inductor not only of photosynthesis, but also of pigment formation.

Characteristic of mutant A-30-1 -- a representative of group V (inertialess) -- is the appearance of maxima in the 680 nm region after light induction (Fig. 1, f). This strain is comparable in its differential spectrum with s. 62, which was no more than to be expected inasmuch as both groups are photoinertialess.

Fig. 1, g shows the spectra of s. g-33. Characteristic of /66
the dark variant of this strain are peaks at 677-682, 435-438 and 410-415 nm, which correspond to the absorption spectrum of chlorophyll, neoxanthin and violaxanthin, respectively, while the absorption spectrum after illumination indicates the loss of the above-enumerated pigments under the influence of light. These data are in sharp disagreement with the respiratory metabolism data for the indicated strain.

The solution to this problem is provided by comparative analysis of respiratory metabolism in mineral and organic media (cf. table). Organic substances in the nutrient medium, as is known, influence the respiratory metabolism of Chlorella. Earlier we showed that this phenomenon depends on the degree of assimilability of the organic substance and its concentration [8]. Moreover, a strain difference, too, should exist. It can be demonstrated on the example of the culture in question. Thus, within the limits of group I, there were: mutants with the same level of respiratory metabolism in mineral as in organic media (for example, s. w-5w); strains in which the intensity of oxygen consumption in the dark and in the light in the presence of organic substance was sharply increased (for example, s. g-34, 43-13); and even organisms whose respiration was substantially activated by transfer to a mineral medium (for example, L_2). Significantly, these data were obtained from parallel measurements of the starting culture, so that they cannot be explained away by variations in different cultivations. But the general tendency

But the general tendency toward inducibility that is characteristic of a given group is kept both in mineral and organic media.

Quite different is the picture in the case of the mutant g-33. Its O_2 absorption in the light drops considerably more intensively (in absolute and relative values) as compared with the same process in a mineral medium. This brings to mind not a culture's transition to photosynthesis under the action of light, but inhibition of the metabolism by light, which is more intensive in the presence of organic substance and, consequently, more responsive to unfavorable influences, which agrees completely with the action of light on the absorption spectrum in the region of green pigments. Thus, we can distinguish still another group of pigments -- photonegative -- on the representatives of which light acts as an inhibitor, leading to a drop in the total level of vital activity (respiration) and individual processes (content of certain pigments). Finally, comparison of the changes in the respiratory metabolism under the action of light points to still another peculiarity. The respiratory metabolism of s. 62 in a mineral medium is characterized, on the one hand, by a pronounced photoinducibility and, on the other, by inertia incident to transition to restoration after shadowing. /67

Introduction of organic substances into the medium depresses photoinduction and, as we may suppose, hinders the reorganization of the metabolism that is necessary for photosynthesis, so that a second characteristic trait is also lacking -- respiratory inertia. Nothing after shadowing can now prevent a sharp increase in the level of O_2 absorption, and this does indeed take place (cf. table) inasmuch as there is a change in neither the metabolism nor the inertia of the transition state.

This relationship does not extend to representatives of the other groups. In particular, in inertialess strains a sharp change in one state does not prevent as sharp a change in another. This may either be connected with a very rapid change in direction of the respiratory metabolism or be independent of this direction, in which case there can be no question of any change at all -- all lighting conditions simply bring about the direction of respiratory metabolism that is already "awaiting" them. /68

These data can help to explain many of the subtle biochemical differences in metabolism, including the changes in the electron transport chain of photosynthesis. I.I. Varasova and K. V. Kvitko [2], Butler [17] and Claes [18] have shown that chlorophylls are lacking in mutants of Chlorella that are highly sensitive to light, and that the qualitative and quantitative composition of the carotenoids is markedly changed, which is corroborated by our experimental data. Disclosure of some groups of mutants by photoinducibility makes it possible to study the fine processes of photosynthesis and respiration in mutants of Chlorella, as has been done by Levine in the case of mutants of Chlamidomonas [19, 24]. /69

Fig. 2. Electron microphotographs (27,000x) of cells of strains of Chlorella vulgaris. Strains: a. 62; b. 14; c. g014.

[Fig. 2, c on following page]

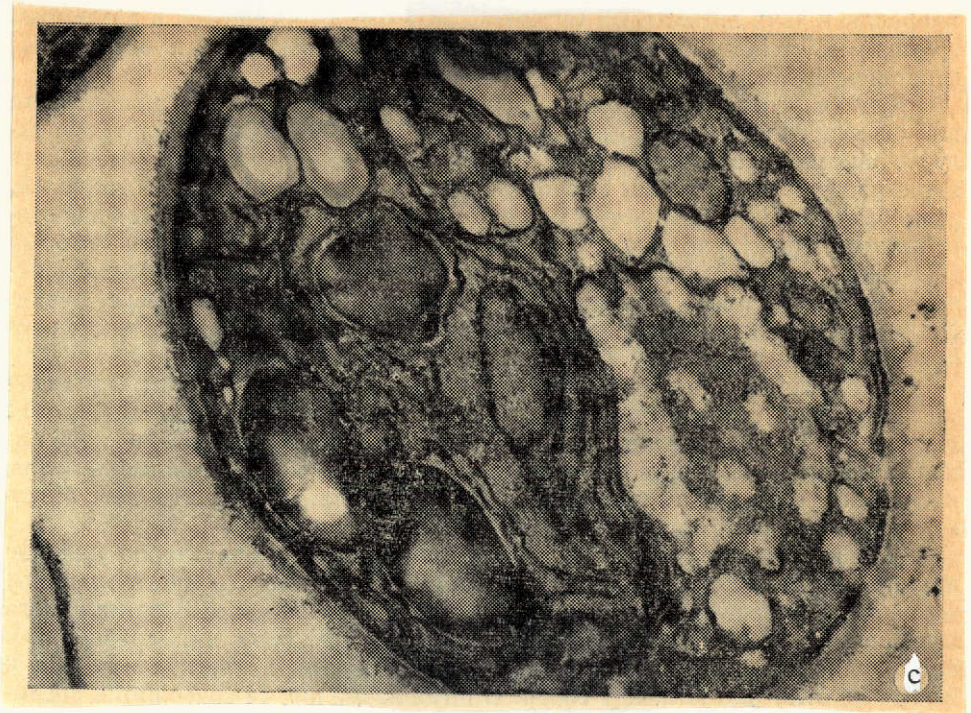

Fig. 2, c.

We believe that the peculiarities found in the strains also make it possible to rephrase the question of how to select cultures for practical purposes. Thus, one of the essential problems connected with the creation of a biological system of safeguarding life is that when an orbital station enters Earth's shadow, not only do photosynthetic organisms cease to liberate oxygen, but they even begin to consume it energetically. This being so, it is easy to see on the example of the inertialess group that the absolute values of O_2 liberation and absorption under appropriate lighting conditions may almost coincide. As a result, "biological regeneration" loses its meaning. In principle, O_2 absorption can change only in the case where cultures will be found that practically do not absorb oxygen in periods of shadowing and actively liberate it right away upon exposure to light. Our experiments point to the possible existence of such cultures. It can be expected that as a result of the search for strains that meet this requirement, cultures with even better characteristics will be found. The prerequisite for this is groups of photo-inertialess and inertia-breathing strains.

In parallel with our study of physiologic peculiarities, we also conducted electron microscopic investigations of the cells of a rich strain of Chlorella and two pigmentational mutants (14, g-14) that belong to different groups in terms of the degree of inertia /70 with which metabolic processes are changed under the action of light and darkness.

The cells of photosynthetically inertialess and inertia-breathing s. 62 of Chlorella have the ultrastructure that is usualy for mesophilic strains, and it is described in the literature [22, 23, 27, 33-35, 37]. The cells contain cup-shaped nuclei, chloroplasts, mitochondria, and some vacuoles whose sizes vary from 0.3 to 0.6 μm (Fig. 2, a). Moreover, characteristic of this strain is the presence of vermiform mitochondria, which may be as long as 3 m, with a great number of [term unknown]. The chloroplast is surrounded by a double membrane and has a lamellar structure. The lamellas cluster in fascicles. In the plastid there is a round body of albuminous nature -- the pyrenoid -- which is often pierced by the lamellas.

The main differences in the ultrastructure of the cells of pigmentational mutants reside in the structure of the plastid. In inertia-photosynthesizing s. 14, the lamellar structure of the plastid is not very pronounced (Fig. 2, b). The number of lamellas in the plastid is inconsiderable. They do not cluster in fascicles; individual lamellas can be observed around globules of reserve material. There is no pyrenoid. The mitochondria have an oval shape, with a small number of [term unknown].

Also characteristic of the cells of photoneutral strain g-14 is the presence of large plastids with individual lamellas and vesicles and numerous globules of reserve material (Fig. 2, c). Nevertheless, the plastids of this strain have a more developed lamellar structure as compared with inertia-photosynthesizing s. 14. The mitochondria in the cells of s. g-14 and s. 14 do not differ from one another. The cytoplasm of s. 14 contains specific reserve material.

Thus, the electron microscopic investigations we conducted of some Chlorella strains belonging to different groups in terms of the degree of inertia of the change in metabolic processes under the action of light and darkness, point to a connection between photosynthetic activity and its inertia and the development of a lamellar structure of the plastid.

Abstract

The authors discuss the possibility of using Chlorella as a simple and convenient model for studying the distinctive features of photoinducibility in organisms with a differentiated nucleus. Comparative studies of respiration, photosynthesis, pigment formation and ultrastructure in different strains showed the expediency of chosing Chlorella as a model subject and allowed some conclusions to be drawn about the selection of strains for oxygen regeneration in closed ecological systems. Thirty-seven references, two figures, one table.

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ON A POSSIBLE MECHANISM OF STABILIZATION OF ALGAL POPULATIONS

N. S. Shevchenko and V. S. Sakoda

Chlorella is one of the promising and widely studied subjects that can be used in creating closed ecological systems. Although the growth and development of the alga's population are very stable, these processes are influenced by a great number of exogenous and endogenous factors, including Chlorella metabolites. To the latter also belong nucleic acids and their degradation products. From this point of view, they have almost not been studied. /71

Recently, in addition to the well-studied genetic effects caused by nucleic acids (transformation, mutagenesis), various phenotypic (nongenetic) effects of compounds of this type have also become known. The action of nucleic acids and their derivatives in stimulating and inhibiting growth and development has been described [1, 10, 17, 18]. Exogenous RNA can cause a temporary change in the metabolic processes of the donee towards the donor organism [3, 8, 16]. Exogenous nucleic acids can exert a regulatory function [14, 20]. The literature contains information about the effect of one DNA preparation on the transformation caused by another. These data are contradictory, but the very fact of an effect is not open to doubt [5, 12]. Also described has been the antiradiation and therapeutic action of nucleic acid preparations [9, 15, 19].

Thus, the wide radius of action of nucleic acids and their derivatives and the liberation of the substances into the surrounding environment in the process of vital activity [13] may be of some moment in the growth and development of a population of microorganisms, including chlorella.

In this connection it would be interesting to analyze the known contradictions between, on the one hand, the frequency of mutation and, on the other, the surprising stability of algal populations growing under continuous conditions. The appearance of mutants does not depend on cultivation conditions. If we assume the most widespread estimate of the probability of mutation per gene to be on the average about 10^{-7} for a generation and a number of genes in each Chlorella cell to be on the order of 10^4 and, finally, that the frequency of appearance of mutants will be a constant value, then, taking into account the daily increment in biomass that is necessary for the safeguarding of one man, we find that 10^{11} mutant cells will appear in the cultivator daily. Even if this calculation errs by an order of magnitude, it does not gainsay the possibility that a form could arise which might /72

replace the original strain because of its greater competitiveness under given conditions. Such a high figure of probable mutants combined with the fact that undesirable changes are in practice absent from mass cultures points to the presence in a population of some mechanisms that prevent the development of mutant forms.

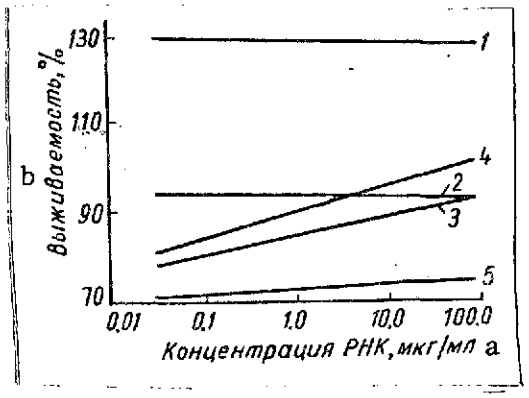

Fig. 1. Effect of high-polymer RNA on the survival of cells of Chlorella mutant g-14: RNA: 1. freshly liberated; 2. preserved 2-3 days; 3. preserved 12-13 day; 4. liberated at 68°C: 5. liberated at 85°C.

Key: a. Concentration of RNA, μg/ml; b. Survival, %;

It is possible that one such factor regulating the population level is some products of the vital activity of algal cells, and, in particular, nucleic acids and the derivatives Chlorella liberates in the process of growing and developing in a medium. It is therefore apposite to study the effect of the nucleic acids liberated from a rich Chlorella strain, and of its degradation products, on the mutants of this alga.

Research methods. As model subject we chose Chlorella's pigmentational mutant g-14 [11]. A cell culture of the mutant strain was grown for 3-4 days in an agared FDGA [2] medium, the products of its own metabolism were washed off twice with 0.14 M NaCl solution with subsequent centrifugation for 3-5 min at 1.5-2000 rpm. In different versions of this experiment, a suspension of such cells (density 2 million/ml) was mixed with a nucleic acid solution of the necessary concentration in different volumes. As control we used a mixture of cells of the investigated subject with a solvent of nucleic acid -- 0.14 M NaCl (in the same proportions). Nucleic acids were obtained from the cells of the rich strain Chlorella vulgaris 62 (s. 62) [6] by the phenol-detergent method [4]. In the investigated preparations, Loury's protein reaction was negative.

The mixtures of cells with nucleic acids were incubated for 2 hours at 33-35°C (with periodic stirring), after which they were sown in a Petri dish on agared FDGA medium (1000 cells per cup). The number of repetitions for each variant was equal to 10. The number of colonies grown was calculated on the fifth-sixth day. The data were processed graphically by the rectilinear compensation method [7].

Discussion. On investigating the effect of exogenous high-polymer RNA obtained from rich s. 62 on the survival of cells of mutant g-14, we detected a dependence of the effect induced on the state of the preparation utilized. Thus, freshly liberated RNA stimulated the growth and development of cells of s. g-14 almost by 30% as compared with the control (Fig. 1, 1). This same preparation, when preserved for 2-3 days, almost lost its activeness (Fig. 1, 2), but on the 12th-13th day it induced in the investigated subject's survival an inhibition that became more pronounced with decreasing concentration from 100 to 0.1 μg per 1 ml (Fig. 1, 3). An inhibitory effect could also be observed when the cells were acted upon by degradation products of high-polymer RNA obtained at 68 and 85°C. The degree of degradation of these preparations was shown by fractionation on MAK columns (Fig. 2, a). These data are shown in Fig. 1, 4 and 5.

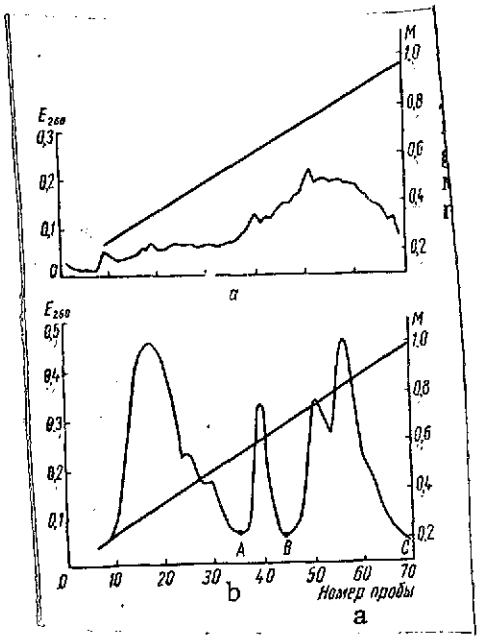

Fig. 2. Chromatographic profile of gross preparation of nucleic acids obtained from cells of Chlorella s. 62 (MAK): a. at 85°C; b. at 0°C; AB - RNA; BC - high-polymer RNA.

Key: c. Specimen No.

It is possible that the investigations we carried out to some extent model processes that come about incident to the growth of an algal culture. Thus, Demain et al. [13] noted that intact cells liberated microorganisms into a medium of high-polymer RNA that began to depolymerize after some lapse of time. The same authors present data indicating that the cells also liberated DNA, which turned out to be stabler in the medium than RNA. In this connection, it seemed apposite to check the phenotypic effect of DNA that was obtained from rich s. 62 on the cells of mutant g-14. To exclude the effect of possible RNA admixtures, we used a DNA preparation that was purified on MAK columns (Fig. 2, b). For comparison we took a high-polymer RNA preparation obtained by the same method. The results, which are shown in Fig. 3, 1 and 2, show that DNA inhibits the survival of mutant cells in proportion to its concentration, while RNA stimulates it, as it did in the preceding experiment with freshly liberated preparation. The data we obtained on the nongenetic action of DNA on mutant Chlorella cells agree with those of other authors, who have shown the acutely ha

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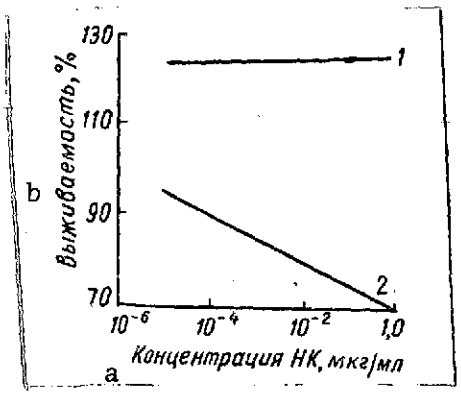

Fig. 3. Effect of exogenous nucleic acids purified on MAK columns on the survival of cells of Chlorella mutant g-14: 1. high-polymer RNA; 2. DNA.

Key: a. Concentration of NA, μg/ml; b. Survival, %

bactericidal (up to a suppression of 93%) action of this nucleic acid on streptococcus cells [18].

Thus, we have shown the variety of the phenotypic action of nucleic acids and their degradation products on the growth and development of cell cultures from a mutant Chlorella strain. If the effect of stimulation can to some extent be explained by a nonspecific action (for example, the presence in RNA of substances of the cytokinine type [10]), the inhibition we observed may indicate that nucleic acids liberated into a medium by cells in the process of their vital activity play a considerable role in regulating the growth and development of algal populations. It must also be taken into account that polymer nucleic acids, after falling into a culture medium, inevitably become so degraded that even fractions that are prone to stimulation will neverthless attain a state in which their inhibitory effect will make itself felt.

Abstract

The authors discuss their own and other data on the many-sidedness of the phenotypic action of exogenous nucleic acids and their degradation products. It is suggested that these compounds may have a regulating role in the growth and development of populations of Chlorella algae. Twenty references, four figures.

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